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REGENERATION OF *PLEUROTRICHA* AFTER MEROTOMY WITH REFERENCE ESPECIALLY TO THE NUMBER OF MICRONUCLEI AND THE OCCURRENCE OF UNINUCLEATE CELLS.[1]

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Lewin,[1] working with *Stylonychia*, has reported a series of experiments in which, after merotomy, he observed an increase in the number of micronuclei in the regenerated merozoa. It was thought to be of interest to repeat Lewin's experiments on another member of the hypotrichus group to determine if there might be any general application of the phenomenon he had observed. Pleurotricha was chosen for the experiments.

Material.

The animals used were secured from a strain, the originator of which was isolated by Dr. George A. Baitsell in the biological laboratory of Yale University. The strain had been adapted to laboratory hay infusion media and was preserved as a stock culture in sterile test-tubes plugged with cotton. Such a culture tube of organisms was given me and from it subcultures were made to other tubes of fresh media and to fresh media in small glass capsules.

The hay infusion used as medium was prepared by placing about 10 grams of field hay in 200 c.c. of tap water in an Erlenmeyer flask and boiling it over a bunsen burner for a few minutes. This medium was made up but once and was used in the proportion of one drop of the infusion to five drops of tap water, this amount of fluid in a capsule serving as the medium for a single animal for twenty-four hours, or till it divided.

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

Method.

Merotomy was performed according to the method of Calkins. The animal selected was drawn up into a fine pointed pipet and placed on a clean glass slide under a Greenough's binocular microscope, eye-pieces 4 and objectives a. The medium was then drawn off till a drop of only sufficient size for the animal to swim in freely was left. With an ophthalmologist's iridectomy knife the animals were cut in parts. The point was ground off the knife and one edge ground to a semi-bellied shape. The blade was plunged into the drop with the animal and the posterior point of its edge allowed to rest on the surface of the slide. As the animal passed from one side to the other of the drop, or around the resting point of the knife edge, successive attempts were made by moving the knife handle up and down to cut the animal as it came directly in line with the edge of the knife.

The frangibility of the cell body afforded one of the first methods of securing fragments of infusoria for study. *Pleurotricha* appears to be very frangible. On one occasion, experiment 48, catching an animal on the surface of the media with a sudden and forcible blast of air from a fine pointed pipet the animal was broken in two. Simply drawing the animal rather forcibly in the pipet was sometimes sufficient to break it in two as in experiment 40. In a few of the experiments the merozoa were secured by drawing the animal selected into a fine pointed pipet and spurting it out forcibly on the side of the capsule.

Animals to be stained were isolated from the capsule with a fine pipet on a clean microscopical slide under a binocular microscope. They were killed and fixed in 5 per cent. glacial acetic acid in saturated mercuric chloride, stained by the Heidenhain iron hematoxylin method, and mounted in xylol balsam.

The Effect of Merotomy on the Number of Micronuclei.

In Tables I. to V., inclusive, are recorded 27 experiments in which the regenerated merozoön was recovered, successfully stained, and mounted. The tables are divided according to the position of the cut. They also state the exact time before or after division, when it had been observed, the length of time after merotomy before the animal was killed, and the number of macronuclei and micronuclei found in the stained merozoön.

In experiments 31, 32, 33 and 57 the animals were killed from 38 minutes to 3 hours and 30 minutes after merotomy. All of these, except experiment 31, were mid-body cuts and it is reasonable to assume that the condition found is normal,—the macronucleus and the micronucleus having not yet divided, or being cells of the uninucleate variety which were also found in control

TABLE I.

ANTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
45	—	9 hrs. 53 min. after cut.	2 macronuclei, 2 micronuclei
105	Before division	27′ 25″ after cut.	2 macronuclei, 2 micronuclei

cultures of this animal. In experiment 57 the micronucleus is in mitosis and the cell appears normal.

In experiment 22, also a mid-body cut, 1 hour and 10 minutes after operation, there are two micronuclei in mitosis. The nucleus is single, swollen and enlarged, as if ready to divide. The most plausible explanation of the condition found here is that the cell originally had two macronuclei and three micronuclei, as was found in certain animals taken from culture.

TABLE II.

POSTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
4	1 hr. 7 min. after division.	3 hrs. 18 min. after cut	2 macronuclei, 2 micronuclei.
15	Before division	6 min. after cut	2 " 2 "
27	"	30 " " "	2 " 2 "
33	"	38 " " "	1 " 1 "
34	"	1 hr. 10 min. after cut	2 " 9 "
39	"	8 hrs. 27 " " "	2 " 2 "
49	"	18 hrs. 7 " " "	2 " 2 "
106	"	27 hrs. 15 " " "	2 " 2 "
107	"	26 hrs. 55 " " "	2 " 2 "
109	"	22 hrs. 36 " " "	2 " 2 "

When merotomy was performed the animal was separated in two merozoa, one of which had a single macronucleus and a single micronucleus; the other merozoön which was saved and which regenerated had a single macronucleus and two micronuclei.

In experiment 34, a posterior-end cut, 1 hour and 10 minutes after operation, we have an abnormal and an interesting animal. The cell is pointed at both ends and broad in the middle, measuring 112 x 45 micra. The anterior macronucleus is situated

TABLE III.

MID-BODY CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
7	1 hr. 17 min. after division.	3 hrs. 15 min. after cut.	2 macronuclei, 2 micronuclei.
22	Before division	1 hr. 10 min. " "	1 macronucleus, 2 "
25	" "	2 hrs. 25 " " "	2 macronuclei, 2 "
31	" "	2 " 30 " " "	1 macronucleus, 1 micronucleus.
32	" "	3 " 30 " " "	1 " 1 "
35	" "	11 " 40 " " "	2 macronuclei, 2 micronuclei.
47	6 hrs. 8 min. after division.	14 " 47 " " "	2 " 2 "
51	6 hrs. 3 min. after division.	13 " 3 " " "	2 " 2 "
57	———	1 hr. 35 " " "	1 macronucleus, 1 micronucleus. (dividing)
88	Before division.	14 hrs. 3 " " "	4 macronuclei, 4 micronuclei.
93	" "	41 " 30 " " "	2 " 2 "
95	" "	45 " 21 " " "	2 " 2 "

nearer the anterior end than normally. It is round in shape, measuring 5 micra in diameter. Its chromatin is homogeneous, deeply stained, showing no vesicles or granules. The posterior macronucleus is likewise situated nearer the anterior end than normally. It measures 7 x 5 micra and its chromatin is of the

TABLE IV.

ANTERIOR- AND POSTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
99	Before division.	43 hrs. 47 min. after cut.	2 macronuclei, 2 micronuclei.

same character as that of the anterior macronucleus. There is one micronucleus adjacent to each macronucleus and seven other micronuclei scattered irregularly throughout the anterior portion of the cell. They are all of about the same size, 2 micra in diameter, deeply stained and homogeneous. The cytoplasm

is finely granular. The changes here appear more like those of a degenerative than those of a physiological process and may have been present at the time the animal was operated upon.

In the twenty other experiments there were two macronuclei

TABLE V.

LONGITUDINAL CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
			(dividing)
86	Before division.	20 hrs. 20 min. after cut.	4 macronuclei, 4 micronuclei.
110	" "	25 hrs. 50 min. after cut.	2 macronuclei, 2 micronuclei.

and two micronuclei, the number normally and most constantly found in this strain of animals.

In Table VI. are recorded the observations made on twelve animals, descendants of merozoa and selected from various generations from the second to the thirty-fifth. In all of these animals there were two macronuclei and two micronuclei.

TABLE VI.

Exp. No.	Cut.	Generation after Cutting.	Number of Macronuclei, Number of Micronuclei.
16	Anterior.	35th	2 macronuclei, 2 micronuclei.
21	Posterior.	30th	2 " 2 "
25	Mid-body.	24th	2 " 2 "
37	Anterior.	10th	2 " 2 "
53	"	2d	2 " 2 "
61	Mid-body.	4th	2 " 2 "
64	Posterior.	2d	2 " 2 "
64	"	3d	2 " 2 "
70	"	2d	2 " 2 "
70	"	3d	2 " 2 "
85	Anterior.	2d	2 " 2 "
106	Posterior.	2d	2 " 2 "

Thus, the evidence furnished by these experiments is that merotomy does not produce any change in the normal number of macronuclei and micronuclei, either in the merozoön or in its descendants as far as the thirty-fifth generation. The occurrence of cells with more micronuclei than macronuclei are more readily explained as having been mechanically produced by the operation itself or as the manifestation of an abnormal cell process that may have existed before merotomy was performed, as such cells are found in normal laboratory cultures of this animal.

The Occurrence of Animals Containing but One Macronucleus.

On four occasions animals from the stock cultures and from the cultures of the control strains when killed and stained were found to possess but one macronucleus and sometimes one and sometimes two micronuclei. These animals were all large slow swimmers or crawlers, and were selected because experience had taught that these were the animals that showed early stages of division, which were being sought at that time.

Among the descendants of merozoön c, experiment 24, a uninucleate animal with two micronuclei was found. The original merozoön was from an animal cut during division. From this it was thought that perhaps injury to the cell during division might be the cause of this occurrence, but out of a large number of animals out of this strain and other strains derived from animals cut during division no other animals with a single macronucleus were found.

In experiment 76 a large slow crawler, evidently near division, was cut anteriorly at 12:25 P.M., August 9. The anterior merozoön disintegrated immediately, the posterior merozoön increased its activity. It was isolated into a clean glass capsule with five drops of tap water and one drop of hay infusion. On August 10, 10:15 A.M., there was found in the capsule three animals, two small and of the same size and a single large animal. The interpretation made of this was that the merozoön had regenerated, divided once into two individuals and that one of the latter had again divided while the other was now approaching division. On killing and staining all three of these animals it was found that each of the small animals had two macronuclei and two micronuclei, but the large animal had only one macronucleus and two micronuclei. The macronucleus appeared about to divide.

Discussion.

Since uninucleate forms occur both among the stock cultures, the control strains and among the merozoa, a conservative inference must be that they are normal variations of this animal, possibly brought about by its being adapted to laboratory media. The relation between the two may be somewhat analogous to

that which Calkins[2] has found to exist between the so-called *Paramecium caudatum* and *Paramecium aurelia*, except that there it is a variation in the number of micronuclei; here, a variation in the number of macronuclei. It appears possible that by proper selection a strain of Pleurotricha may be obtained in which all of the animals, for a period, may show only one macronucleus.

From the experiments it appears fairly conclusive that merotomy generally has no effect on the normal number of micronuclei. Animals with less macronuclei than the normal may be found both in laboratory cultures of this animal and among the descendants of merozoa. Animals with more than the normal number of micronuclei may occur in laboratory cultures. The single instance in which there was found among the merozoa an animal with two abnormal macronuclei and several micronuclei is undoubtedly that of a degenerative or a necrobiotic cell. This is somewhat suggestive that multiple micronuclei may in general be one of the manifestations of a degenerative or a necrobiotic process in the cell.

It is to be noted that these observations are not in accord with Lewin's findings for *Stylonychia*. It appears from his paper that he had some difficulty in getting his animals to survive in the media used and another possible explanation for his observations is that they were more the results of necrobiosis and degeneration in the culture strains than the effect of merotomy.

Conclusions.

1. Merotomy has no effect other than the effect that may be mechanically produced by the operation itself on the number of micronuclei of *Pleurotricha*.

2. Animals with more than the normal number of micronuclei and less than the normal number of macronuclei may occur in laboratory strains of this animal as well as among merozoa.

Acknowledgments.

I am indebted to Dr. G. N. Calkins for suggesting this study to me and for assistance, also to Dr. George A. Baitsell for the

cultures of *Pleurotricha* with which the work was performed. To each of these men I desire to express my sincere appreciation.

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THE REACTIONS OF CRAYFISHES TO GRADIENTS OF DISSOLVED CARBON DIOXIDE AND ACETIC AND HYDROCHLORIC ACIDS.[1]

EDWIN B. POWERS.

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I. Introduction.

The following experiments were undertaken to determine the reactions of crayfishes to gradients of acids, and, if possible, to determine the relation of the distribution of carbon dioxide contained in water to the natural distribution of the crayfishes. It was also hoped that something might be added to the present knowledge of the physiology of rapid modification of animals in

[1] From the Hull Zoological Laboratory, University of Chicago.

gradients. The experiments were conducted between January 13 and April 27, and between June 16 and July 31, 1913. The first set will be designated as low-temperature experiments and the second set as high-temperature experiments.

II. Material and Methods.

1. *Apparatus and Method of Experimentation.*

In the study of reactions of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids, the method and apparatus were devised by Shelford and Allee ('13) for the study of reactions of fishes to gases or solids in solution. The apparatus, a full description of which is given in Shelford and Allee ('13) "The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases" (pp. 225–229), consists of two tanks each 120 cm. long by 20.5 cm. wide by 14 cm. deep with outlets at the center of both sides, near the top. These outlets are guarded by a screen-bottomed tube which extends across the tank. The tanks are placed side by side in an aquarium, beneath a hood under identical and symmetrically surrounding conditions. Tap water was introduced at both ends through perforated tees behind screens. In the experiments with carbon dioxide, the flows were 600 c.c. per minute. At one end carbon dioxide was introduced into the inlet so that a gradient was produced lengthwise of the tank between the tap water and the water high in carbon dioxide. This was shown by titrations made of samples taken from different portions of the tank. In the acetic and hydrochloric acid experiments the conditions were the same, except for the flow at the tap water end and corresponding end of the control, which was 1,200 c.c. per minute, thus producing a sharper gradient. This flow was also used in all high temperature carbon dioxide experiments. The acetic and hydrochloric acids were introduced by means of a separate tee.

After everything was made ready for the keeping of records, the crayfishes were dropped into the center of the tank and were observed through a slit in the hood, and the back and forth movements recorded in the form of a graph on paper especially prepared for this purpose. Records were also kept of specific reactions observed in any individual. These records were made

between the spaces set aside for the graphs. The previous history of the animals and the gradient used with a description of conditions of experimentation were entered at the top of the page. After the crayfishes were dropped into the middle of the tank they were disturbed as little as possible. At the end of each experiment a titration of samples of water taken from the two ends and center was made with sodium carbonate and recorded.

2. *Stock.*

The crayfishes were of the species *Cambarus propinquus* Gir., *C. virilis* Hag., *C. diogenes* Gir. and *C. immunis* Hag. They were all of medium size with the exception of a few specimens of *C. virilis* and *C. diogenes* which were above medium size. *C. propinquus* and *C. virilis* were all taken at New Lenox, Ill., from the pools just above and just below the rapids and from among the rocks of the rapids of Hickory Creek. The first stock was obtained December 25, 1912, and was kept in the laboratory in a large pan with the bottom covered with sand, gravel and some vegetation. The water was changed six times per week. When brought in a few died during the first three to five days, due possibly to the sudden change of temperature, but after this there was little mortality. Stock obtained November 15, 1912, and January 25, 1913, which were kept in an aquarium, suffered the same experience when transferred to the pan. Stock obtained March 22 and 29 and June 21, 1913, was kept in an aquarium in which was a strong flow of water to lower the temperature to more nearly that of experimentation. These suffered no great mortality. *C. immunis* was obtained from sloughs, mostly from the bottom, and a few from burrows, and *C. diogenes* from burrows only, along the banks of the same sloughs near Clark Junction, Ind., June 20 and 24 and July 9 and 26. These were all kept in large glass jars into which fresh water was flowing. There was no great mortality experienced by these stocks.

3. *Habitat.*

C. propinquus is essentially a quiet water stream form. It inhabits the pools with more or less muddy bottom (Williams, '01). They hide under rocks or rest quietly on the bottom.

Sometimes they lie concealed in short burrows along the banks (Harris, '03). *C. virilis* is found to be absent from the muddier and shallower portions of the streams but is sometimes taken in ponds with *C. immunis* (Harris, '01), but is more often found in running streams, among the rocks of the more rapid portions (Harris, '03). They do not burrow except when the ponds begin to dry or winter approaches. Garman ('89) took this species from wells, Wilson's cave and streams. *C. diogenes* is a burrowing species and often makes mud chimneys, often burrowing in damp ground some distances from the open water, which it seldom enters except during the breeding season (Pearse, '10). *C. immunis* is a mud-loving species and is found mostly in small pools, though it sometimes occurs in brooks and rivers (Pearse, *l. c.*) (Harris, '03).

4. *Senses.*

The fact that crayfishes are sensitive to chemicals has been shown by several authors. Putnam ('75) noted that *C. pellucidus*, the blind crayfish, will hunt food when it is thrown into the water. Holmes and Homuth ('10) found that the whole body is more or less sensitive to olfactory stimuli, and that the antennæ, mouth parts and tips of the chelipeds are sensitive in the order named. Nagel ('94) observed the chemical sense in *Astacus*. Wright ('84), by a study of the antennules of *C. propinquus*, found that five of the eighteen segments, *i. e.*, eleven to fifteen inclusive, bear eight of the so-called olfactory organs and the distal nine fewer. Bell ('06) found that the crayfishes with which he worked reacted positively to meat juice and negatively to lavender water, acids and salts, and concluded that they are sensitive over the entire body but more in the anterior appendages than in other parts. Chidester ('12) found when meat was thrown into the water that crayfishes would approach and seize fresh meat quicker than they would meat dried in the air.

III. The Sensing of Carbon Dioxide and Acetic and Hydrochloric Acids.

The crayfishes sensed the carbon dioxide and acetic and hydrochloric acids when passing into the high acid concentration end of the experiment tank. This was indicated by certain specific

reactions which were made as the crayfishes entered the higher concentration. Such reactions were waving the antennæ, moving the appendages, backing and walking with the legs extended. The waving and moving of the appendages were especially noted in the reactions of *propinquus*, *virilis* and *diogenes*, in the carbon dioxide experiments in which the concentrations of carbon dioxide in the two ends were 40 to 60 and 80 to 100 c.c. per liter respectively. The animal would also crouch down in the corner of the tank. This tendency to wave the antennæ and move the appendages was present in *virilis* in the acetic acid also. The backing reaction was not so common in the carbon dioxide experiments but was marked in the hydrochloric and acetic acid experiments.

IV. The Effect of Carbon Dioxide and Acetic and Hydrochloric Acids.

1. *The Effect on Reflex of the Crayfishes.*

The crayfishes not only detected the presence of the acids but were intoxicated or anæsthetized by them in the low temperature experiments, possibly due to the less regular movements of the animal. This is shown in graphs (Chart I.) by the longer periods of time required to cross the tank in the experiment than that required in the control. The effect was greatest in the experiments with high concentrations. The first effect of the carbon dioxide was to interfere with the correlation of movements and to cause the animal to carry the body high with legs extended. Progressively locomotion became slower and slower until it ceased, but the appendages were still moved one after the other. The crayfish would finally fall upon its back and continue to move its appendages for a short time, after which it would remain motionless as if dead. It would recover rapidly if placed in fresh water and after a short time move about normally. The acetic acid produced this same effect upon *virilis* but *propinquus*, *diogenes* and *immunis* were either not intoxicated by the acetic and hydrochloric acids or acted rapidly enough to get out of the high concentration before being greatly affected, (see Charts I. and II.). The movements of *virilis* were always more or less irregular. One of the individuals in experiment 21

was completely anæsthetized but recovered in eight minutes when placed in fresh water. At first it remained motionless in the fresh water but later revived and seemingly became normal.

2. *Anæsthesia and Death.*

An experiment was performed to determine the relative resistance of the four species of crayfishes to high concentration of carbon dioxide. Though the experiment was terminated before the crayfishes had all been killed, through an accident to the apparatus, it had been carried sufficiently far to determine the susceptibility of the four species to the carbon dioxide solution. The apparatus consisted of large glass bottles (Wells, '13) through which water containing from 6.92 to 7 c.c. of oxygen per liter and varying amounts of carbon was flowing. The amount of the gases were determined by titrations of samples of water collected from the over-flow. The temperature was 21.5° to 23° C.

It was found that in all cases the smaller individuals of a species died first. This was probably due to the greater proportion of surface to mass in the smaller specimens than in the larger ones, rather than a difference of susceptibility of the smaller specimens to the carbon dioxide solution. These are the same general results obtained by Wells (Wells, *l. c.*) with fishes.

All remained active in 50 c.c. carbon dioxide per liter. *Virilis* was anæsthetized by solutions of 120 to 145 c.c. per liter. *Propinquus* was not overcome but showed that they were effected, while *diogenes* and *immunis* showed the effect to a less extent. *Propinquus* was not anæsthetized as early as *virilis* but the time of death approached that of *virilis*, the small specimens of *propinquus* having died before the large *virilis*, but a medium sized *propinquus* survived all the specimens of *virilis*. *Diogenes* and *immunis* were much less susceptible to the carbon dioxide than either *propinquus* or *virilis*. All specimens of both *propinquus* and *virilis* died before the medium-sized specimen of *diogenes*. The specimens of *diogenes* and *immunis* died in the following order: One medium-sized *diogenes*, one *immunis*, one *diogenes*, one *immunis*, two *diogenes* and two of *immunis*. There were one *immunis* and one large *diogenes* alive when the experi-

ment terminated. All the specimens of *immunis* were smaller in size than the smallest specimen of *diogenes*. From this data it seems the four species are susceptible to high concentrations of carbon dioxide in the following order: *virilis*, *propinquus*, *diogenes* and *immunis*.

V. The Reaction and Modification in Gradients.

The reaction of crayfishes in gradients is shown in Tables I. and II. Table I. for carbon dioxide, II. for acetic and hydrochloric acids, are arranged in order of sharpness of the gradient, *i. e.*, the difference between the concentrations of acid at the two ends. The four species are grouped separately in each table. Reaction is shown by the time preference for one end or the other, by turnings and by crossings of the center. There is also tabulated the modification of behavior by turnings accompanied by backing. The backing reaction is not indicated in the ratings (Table III.), which is the numerical expression of avoidance of ends when turnings and time spent in the halves of the tank are considered. The ratio of the increased concentration of the acid of the high end over that of the low end, or Weber's ratio, is tabulated for comparison with the difference of concentration of the acid at the two ends.

The crayfishes, when passing into the high concentration end of the tank, gave certain definite avoiding reactions, when not too much affected by the presence of the acid. Of these the reactions recordable in graphs are (1) turning upon encountering gradient, either the first time or after one or more invasions and (2) reactions which cannot be recorded graphically are turning accompanied by backing and crawling on the screen, out of the water, or attempting to crawl on the sides of the tank. Recordable reactions were in the most cases rhythmic and represented a rapid modification of behavior. Reactions of the first class are grouped in one column of the table, but where backing accompanied turning separate mention is made of this fact. Of the reactions that could not be graphically recorded, that of crawling on the screen is least definite as an avoiding reaction since it was noted in the controls also, though less than in the experiments. This reaction is probably due to thigmotactic

TABLE I.

Showing reaction of crayfishes in a gradient of carbon dioxide. In all experiments and controls three animals were tested simultaneously except in the 20-minute experiments and controls in which case each of the three individuals were tested separately, 20 minutes each. H indicates high carbon dioxide and L low carbon dioxide. Corresponding ends of control tank are designated by the same letters. Typical experiments, only, are given in table. Totals and per cent. are totals for all experiments and controls of each species of crayfishes in each gradient. The experiments of each species are grouped and arranged in order of sharpness of gradient between the two ends of tank. For gradient see Table III.

Experiment No.	*Cambarus* Species.	Carbon Dioxide in c.c. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
2	*Propinquus*..	3.5	20.	27.9	72.1	51.	49.	23	40	17	4	3	2	5	0	0	1	6.	60
26	"	3.6	23.	21.3	78.7	37.1	62.9	48	36	31	7	7	12	0	0	0	0	17.5	40
5	"	47.4	94.	5.6	94.4	70.6	29.4	7	41	12	1	1	5	4	0	0	0	3.75	60
	Totals.....							157	237	124	57	32	40	20	3	3	1		
	Per cent.....									71	29	44.5	55.5						
24	*Virilis*.....	1.5	9 5	41.7	58.3			39		30	23			2	0			19.	40
27	"	2.9	46.5	11.1	88.9	24.1	75.9	40	18	40	21	14	20	1	0	0	0	17.5	40
	Totals.....							108	85	78	54	27	24	4	0	0	0		
	Per cent.....									59	41	53	47						
44	*Diogenes*.....	2.3	18.9	33.3	66.7	35.8	64.2	18	21	7	5	5	3	0	0	0	0	20.5	20
28	"	56.5	179.4	26.	74.	58.8	41.2	20	32	18	11	4	3	8	2	0	1	17.5	20
	Totals.....							58	85	69	45	39	29	15	6	0	1		
	Per cent.....									60	40	57.3	42.7						
45	*Immunis*.	2.3	9.4	33.7	66.3	48.3	51.7	52	38	13	5	7	8	0	0	0	0	21.	20
46	"	2.7	24.5	48.5	51.5	54.6	46.4	37	37	13	11	1	2	1	2	0	0	20.5	20
	Totals.....							139	65	49	33	8	10	2	3	0	0		
	Per cent.....									59.8	40.2	44.5	55.5						

response as well as an avoidance of the acids. The tendencies to crawl out of the water and upon the side of the tank are more clearly acid avoiding reactions. In the controls there were no attempts to crawl on the sides of the tank and few animals showed an inclination to crawl out of the water.

1. *Carbon Dioxide Gradient.*

(*a*) *C. propinquus.*—The crayfishes, upon invading the high carbon dioxide end, showed a tendency to turn before reaching the screen, or they would back a short distance and then turn and pass to the lower carbon dioxide end. Of all the individuals tried 55 per cent. turned back on the first encounter of the gradient and 14.8 per cent. of these turnings were accompanied by backings (Table I.). There was a greater number of turnings from the high concentration end than from the low, there being a total of 124 from the high end to 51 from the low. The fact that turning from the high end is an avoiding reaction is emphasized by 16 per cent. of the turnings being accompanied by backing. There were fewer crossings of the center in the experiments than in the controls, in the latter there being a tendency to travel the entire length of the tank. In some cases the reduction of crossings of the center represents the extent of anæsthetization of the animals.

In the experiments with low concentrations (low 3.5 and high 20 c.c. of carbon dioxide per liter) and low temperature the turnings were rhythmical (Chart I., Expt. 2). Long invasions of the high concentration end were followed by shorter invasions and periods of rest at the low concentration end. These were then followed by very short invasions and very long periods of almost complete rest in the low concentration end. Later there would be a second similar period of invasion and rest. Three such periods are shown in the graph. Thus there is a period of increased sensitiveness or a period of increased reaction to the same incoming sensation, after invasion of the high concentration end. In either case there is a rapid modification of behavior.

With higher concentrations (24–47 c.c. per L., low end and 58–94 c.c. per L. high end), if the crayfishes were not too greatly overcome, there was greater rapidity of reaction and a more

TABLE II.

Showing reaction of crayfishes in gradients of acetic and hydrochloric acids. For further explanation see Table I.

Experiment No.	*Cambarus* Species.	Acetic Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
14	*Propinquus*..	.24	1.39	3.5	96.5	54.9	45.1	14	22	15	3	11	13	7	0	0	0	4.25	60
13	" ..	2.41	3.66		100.	61.3	38.7	0	16	4	0	6	6	2	0	0	0	4.25	60
	Totals.....							45	93	81	15	33	34	33	0	1	0		
	Per cent.....									84.5	15.5	49	51						
21	*Virilis*.....	1.21	1.67	41.	59.	45.3	54.7	11	13	17	7	11	5	9	1	1	0	6.25	40
30	"	.266	3.926	3.2	96.8	28.2	71.8	11	15	22	6	6	7	7	0	0	2	16.5	40
35	"	2.88	28.99	23.2	76.8	53.8	46.2	11	24	13	2	6	1	11	1	2	0	17.	20
	Totals.....							101	139	113	46	51	40	52	7	3	5		
	Per cent.....									71	29	36	44						
31	*Diogenes*.....	.086	.312	10.	90.	63.9	36.1	21	27	15	2	11	5	3	0	1	1	16.5	20
33	"	.014	.354	41.1	58.9	92.	8.	26	10	19	10	18	2	5	3	4	0	16.5	20
	Totals.....							93	117	51	29	43	24	14	10	5	2		
	Per cent.....									64	36	64	36						
37	*Immunis*....	.078	.429	29.4	70.6	53.8	46.2	21	45	24	8	11	9	7	2	0	0	18.	20
34	"	.429	1.437	16.7	83.3	54.1	45.9	6	3	5	5	1	3	2	0	0	0	16.5	20
	Totals.....							33	52	34	15	14	16	10	3	0	1		
	Per cent.....									67.4	32.6	46.7	53.3						

TABLE II.—*Continued.*

Experiment No.	*Cambarus* Species.	Hydrochloric Acid in g. per L.		Per Cent. of Time Spent in Halves of Experimental and Control Tanks.				Number of Times Crossed Center.		Number of Times Turned in Gradient from High and Low Ends.				Number of Turnings Accompanied by Backings.				Temperature, Centigrade.	Time of Experiment.
				Expt.		Cont.				Expt.		Cont.		Expt.		Cont.			
		L	H	H	L	H	L	Expt.	Cont.	H	L	H	L	H	L	H	L		
39	*Propinquus*..	.0061	.0157	9.4	90.4	60.1	39.9	29	30	27	6	1	2	4	0	0	2	20.5	20
47	" ..	.0275	.0932	4.7	95.3	49.6	50.4	28	63	39	2	0	1	3	0	0	0	21.	20
	Totals.....							57	93	66	8	1	3	7	0	0	3		
	Per cent.....									89.2	10.8	25	75						
41	*Virilis*.....	.0075	.067	12.4	87.6	49.3	50.7	11	32	24	8	2	6	14	2	0	2	20.5	20
	Per cent.....									75	25	25	75						
42	*Diogenes*.....	.0023	.0078	30.5	69.5			39		7	5			2	0			21.	20
43	"	.0053	.0702	13.8	86.2	39.3	62.7	18	49	14	6	11	13	2	0	0	3	21.	20
	Totals.....							57	49	21	11	11	13	4	0	0	3		
	Per cent.....									66.7	33.3								
40	*Communis*...	.0014	.0304	31.5	68.5	49.3	50.7	23	42	18	7	10	8	2	1	0	1	20.5	20
	Per cent.....									72	28	44.5	55.5						

TABLE III.

Showing the vigor of reaction or rating of the crayfishes in gradients of carbon dioxide and acetic and hydrochloric acids. The rating is obtained by subtracting the percentages given for the time preference for the two ends and subtracting the percentage of turnings from the two ends and adding the two remainders (which are considered of different signs since turnings are from opposite end to end preferred) and dividing by two. The table also shows difference in concentration between the two ends and the ratio of increase of gradient at high end over low end or Weber's ratio.

Experiment No.	*Cambarus* Species.	Grams per L. of Acid or c.c. per L. CO_2.		Difference in g. or c.c. per L. between Two Ends of Tank.	Weber's Ratio.	Rating.		
		L	H			Expt.	Cont.	
22	*Propinquus*	1.5	11.8	10.3	6.8	7.		
2	"	3.5	20.	16.5	4.7	53.	9.	
26	"	3.6	23.	19.4	5.4	60.	26.	
8	"	120.	149.	29.	.24	32.	59.	
3	"	24.	58.	34.	1.4	40.	23.	Carbon dioxide gradient.
5	"	47.4	94.	46.6	.98	86.0	53.	
4	"	43.	90.	47.	1.09	+5.5	20.	
6	"	32.	82.	50.	1.5	32.	59.	
29	"	37.1	154.7	117.6	4.3	42.	32.	
24	*Virilis*....	1.5	9.5	8.	6.3	24.		Ratio of increase of gradient at high end over low end or Weber's ratio.
10	"	18.	54.	36.	2.	+43.	46.	
11	"	62.5	101.	38.5	.26	2.	54.	
27	"	2.9	46.5	43.6	15.	54.	17.	
9	"	86.	172.	86.	1.	38.	3.	
44	*Diogenes*...	2.3	18.9	16.6	7.6	24.	27.	
25	"	3.3	35.9	32.6	9.9	13.	1.5	
28	"	56.5	179.4	122.9	2.1	36.	1.	
45	*Immunis*. .	2.3	9.4	7.1	3.	38.	2.	
23	"	1.3	9.6	8.3	6.4	+ 4.		
46	"	2.7	24.5	21.8	7.7	4.	21.	
17	*Propinquus*	.045	.183	.138	3.2	79.	25.	
16	"	.045	.3	.255	5.7	83.	9.	
15	"	.24	.69	.45	1.9	65.	7.	
14	"	.24	1.39	1.15	5.2	79.	8.	
13	"	2.41	3.66	1.25	.48	100.	11.	
18	*Virilis*....	.018	.114	.096	5.1	53.	11.6	
19	"	.018	.22	.202	11.3	52.	25.	Acetic acid gradient.
21	"	1.21	1.67	.46	.3	39.	8.6	
20	"	.122	.8	.67	5.5	15.	10.	
30	"	.266	3.926	3.66	13.7	75.	21.	
35	"	2.88	28.99	26.11	9.	63.	16.	
31	*Diogenes*...	.086	.312	.226	18.5	78.	1.4	
33	"	.014	.354	.3406	23.8	24.	92.	
38	"	1.027	2.938	1.911	18.	.6	2.2	
36	"	4.97	23.33	18.36	3.6	8.	4.7	
32	*Immunis*. .	.0109	.1105	.0996	9.1	38.	20.	
37	"	.078	.429	.351	4.5	45.	1.2	
34	"	.429	1.437	1.008	2.3	33.	29.	
39	*Propinquus*	.0061	.0157	.0096	1.6	72.	26.	
47	"	.0275	.0932	.0657	2.3	90.	45.	
41	*Virilis*....	.0075	.067	.0595	7.9	63.	12.	
42	*Diogenes*...	.0023	.0078	.0055	2.4	28.		Hydrochloric acid gradient.
43	"	.0053	.0702	.0649	12.2	56.	7.	
40	*Immunis*..	.0014	.0304	.029	20.2	40.	6.	

rapid modification of behavior (Chart I., Expt. 5). Periodicity is still present but is marked by shorter invasions of the high end. This periodicity is better shown by individuals than by groups. The graph of experiment 5, Chart I., shows that there was a complete cessation of invasions of the high end after 48 minutes and the crayfishes were still resting in the low end after 80 minutes. Numerically expressed, 70 per cent. of all individuals tried showed modification.

(*b*) *C. virilis.*—*Virilis* oriented less definitely to the carbon dioxide gradient than *propinquus*. (Compare ratings, Table III.) The lack of orientation is shown by the percentage of turnings from the two ends and the time preference for one end or the other. This staggering is possibly due to the somewhat more concentrated solutions used, but may be explained as has been suggested by Shelford and Allee ('13) for swift water fishes. That is swift water fishes which encounter very little carbon dioxide may react less definitely to it than fishes which live more often in the presence of carbon dioxide. *Virilis* showed a tendency to crawl out of the water.

(*c*) *C. diogenes.*—*Diogenes* reacted less intensely to both the lower and higher concentrations of carbon dioxide in gradients than either *propinquus* or *virilis*. In experiment 44 with 18.9 c.c. carbon dioxide per liter at the high end there was no backing accompanying the turnings, while in experiment 28, with 179.4 c.c. carbon dioxide per liter at the high end, there were 44 per cent. of the turnings accompanied by backing and one of the turnings on first encounter was accompanied by backing. *Diogenes* also showed a marked tendency in the high concentration carbon dioxide gradient to move forward and then stop after which it would move forward again.

(*d*) *C. immunis.*—All experiments with *immunis* were with concentrations not running above 25 c.c. carbon dioxide per liter at the high concentration end. The avoiding reactions with the exception of experiment 45 were very low. There was a slight positive reaction in experiment 23 as is shown in Table III. There were two turnings from the high concentration end accompanied by backing in experiments 23 and 46 while at the same time there were 3 from the low end.

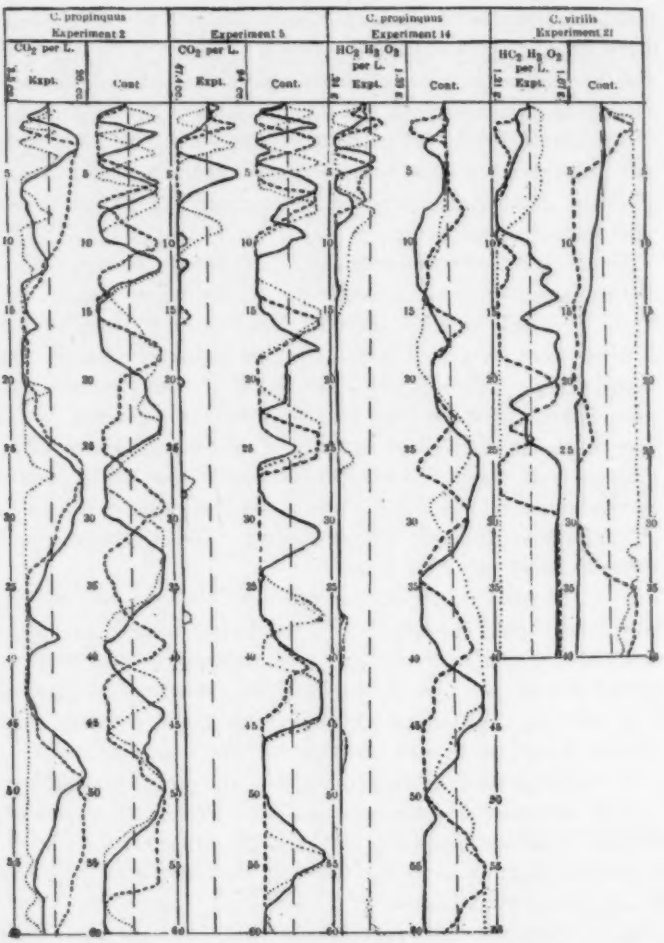

CHART I. Showing reaction of the crayfishes of gradients to carbon dioxide and acetic acid. The horizontal distance between the scales represents the length of the tank. The vertical scale represents time in minutes. Different tracings represent individual animals. Three animals were tested simultaneously.

2. *Acetic Acid Gradient.*

(*a*) *C. propinquus.*—The avoidance of the acetic acid in gradient was sharp and definite (see Chart I., Expt. 14). Orien-

tation was also definite. There seemed to be an acceleration of all the reactions shown in the carbon dioxide experiments. Out of fifteen trials fourteen (93.3 per cent.) showed turning at first encounter of the high concentration, and of the fourteen, four started toward the high end without first having invaded the low or even a portion of it; 50 per cent. of the turnings were accompanied by backing. Only two individuals reached the screen in the acetic acid end. Of these two one crossed after handling; the other occurred in the lowest concentration of acetic acid used. There was, after a certain period of time, a cessation of invasions of the high acid concentration end; this period varied inversely as the total concentration of the acid. Experiment 14 varies slightly from this rule. There was also periodicity of invasions of the high concentration end (see Chart I., Expt. 14) as described in the carbon dioxide experiments.

(*b*) *C. virilis*.—These experiments can be divided into three groups, a low concentration, a high and a very high, *i. e.*, experiments 18 and 19 with a concentration of .0185 g. of acetic acid per liter at the low end and .1145 to .228 g. per liter at the high end; experiments 20 and 21 with .122 to 1.21 and .8 to 1.67 g.; and experiment 35 with 2.88 and 28.99 g. per liter of acetic acid in the low and high ends respectively.

In the low concentration experiments at low temperature there was a more definite orientation and a greater time preference for the low end. This is shown by the ratings (see Table III.). There was also a more or less periodicity of invasions of the high end with a complete cessation of invasions in experiment 19 after 45 minutes.

In the high concentration experiments at low temperature there was less orientation except in turning at the first invasion of the high end. There was but one individual (Expt. 20) that showed periodicity of invasions of the high end. The lack of orientation is shown by graph Expt. 21, Chart I. There was an increased tendency to crawl on the sides of the tank and out of the water. Thus there was a falling off of orientation and a substitution of crawling out of the water.

In experiment 30, in which the temperature was high and specimens above medium size were used, there was better orienta-

tion with a greater intensity of avoiding reactions as is shown by the rating and per cent. of time spent in the halves of the pan. See Tables II. and III., and Chart II., graph 30. While in experiment 35 where the concentration was very high there was a falling off of the intensity of the avoiding reaction due to one specimen becoming more or less anæsthetized. See Table II. and Chart II., Expt. 35.

(*c*) *C. diogenes.*—In the acetic acid experiments as well as the carbon dioxide experiments the intensity of the avoiding reactions are rather low with the exception of experiment 31 which is rather high, the rating being 78. There was a stronger tendency to stop and then move forward and with longer periods of rest than was noted in the carbon dioxide experiments.

(*d*) *C. immunis.*—The avoiding reactions of *immunis* to acetic acid was definite in the time spent in the halves of the pan, by turnings and by turnings accompanied by backings. See Table II., Chart II., Expt. 37 and 34. The intensity of the reactions in the acetic acid as well as in the carbon dioxide was rather low as is shown by Tables I. and II.

3. *Hydrochloric Acid Gradient.*

After having completed the experiments with carbon dioxide and acetic acid it was thought advisable to test the crayfishes with some inorganic acid and thus determine the difference or similarity of the reactions of the crayfishes to the different acids and to better compare the reactions of the four species of crayfishes. Hydrochloric acid was selected for this purpose. Since the ion constant of hydrochloric acid in very weak concentrations is approximately one (Stiegletz, '11) very low, concentrations were used in all the experiments. By an inspection of the tables it is seen that the same intensity of avoiding reaction was obtained in the low concentrations of hydrochloric acid but of high ion concentration as was obtained by the higher concentrations of acetic acid and carbon dioxide of lower ion concentration. Not only was the intensity of the avoiding reactions high in proportion to the concentration, but the reaction of all four species was more definite as is indicated by the turnings from the ends and turnings accompanied by backings. See Table II. and Chart II. Expt. 47, Chart II. was extended over a period

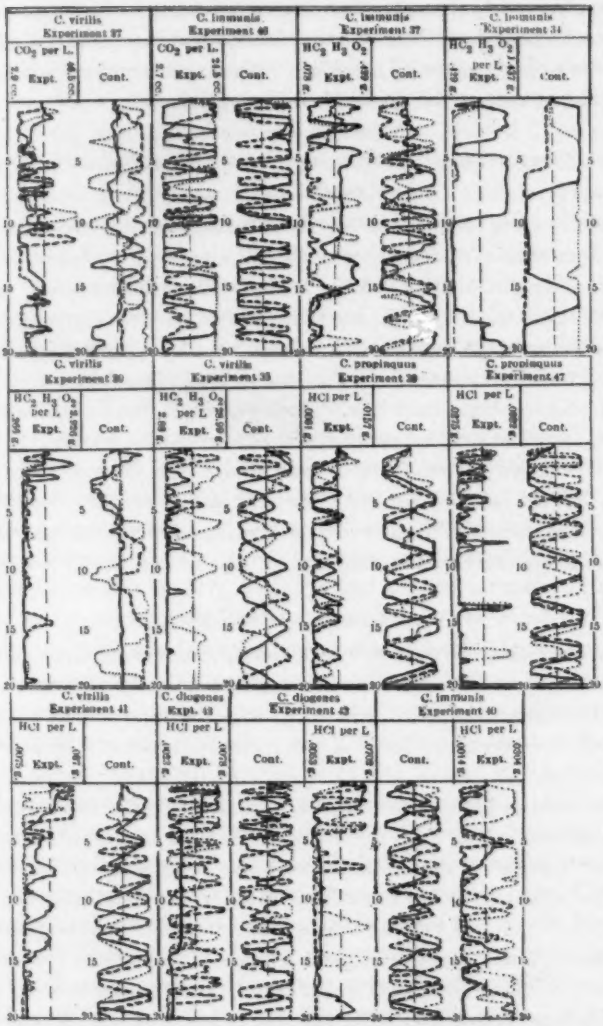

CHART II. Showing reaction of the crayfishes to gradients of carbon dioxide and acetic and hydrochloric acids. The horizontal distance represents the length of tank. The vertical distance represents time in minutes. Different tracings represent individual animals. In all experiments except experiments and controls 27 and 47 each animal was tested separately, 20 minutes each.

of forty minutes to determine the modification of behavior over a longer period of time. It was found that there was a similar rhythm of invasions of the high concentration end with a final coming to rest at the low acid concentration end.

4. *A Comparison of the Reactions of the Four Species of Crayfishes Tested.*

While there were noted specific differences in the reactions of the four species of crayfishes tested, all sensed the carbon dioxide and acetic and hydrochloric acids. Observations show that *propinquus* gives specific reactions and orients to a gradient of these substances, while *virilis* orients to a less degree; *immunis* to high concentrations, and *diogenes* to a still less degree to both high and low concentrations. Both *propinquus* and *virilis* which were tested at low temperatures were affected by carbon dioxide to the extent of intoxication, and *virilis* was affected more or less by the acetic acid. All species, so far as their avoiding reactions were noted, showed modifications of behavior and with *propinquus* there was a tendency to come to rest in the low end.

VI. General Discussion.

In reviewing the data it is seen that in each set of experiments all the types of behavior are increased in intensity with increase in the concentration of acid used, and the question as to the relation between the cause of the different types of behavior is suggested.

In the first place there must be a gradient before there can be orientation. This is in accord with Weber's law which states: "The increase of the stimulus necessary to produce an increase of the sensation bears a constant ratio to the total stimulus,"[1] *i. e.*, there must be a definite ratio between the increased intensity of the stimulus and the original stimulus before there can be a sensation of an increased stimulus. If the crayfishes were reacting in accordance with this law, the rating, which is the numerical expression of the degree of the reaction when both turnings and time preference are considered, should be in proportion to Weber's ratio, see Table III. By turning to the experiments

[1] James, The Principles of Psychology.

with *propinquus* in carbon dioxide and comparing the ratios of the increased concentrations at the high acid concentration end with the concentration at the low end of the low temperature experiments, it will be seen that there is no definite relation between the two. This comparison may be objected to on the grounds that *propinquus* was intoxicated by the carbon dioxide, but by turning to the acetic acid experiment, where *propinquus* was not affected there is seen the same variation between the ratings and Weber's ratio. At the same time it will be seen that there is a more definite relation between the rating and the total concentration of the acetic acid. In general the lowest total concentrations of acids have the lowest ratings. Experiments 15 and 16 are exceptions but still the range is not wide as compared with the great variation of Weber's ratios.

Now turning back to the carbon dioxide experiments (Table III., Expts. 2, 26 and 5) the intensities of the reactions are in reverse proportion to Weber's ratios, but are in direct proportion to the concentrations of the carbon dixoide solution used in the experiments. In the hydrochloric acid experiments (see Chart II. and Table III., Expt. 39 and 47) the intensity of the reactions conform with Weber's ratio, but at the same time it conforms with the concentrations of the hydrochloric acid used. Thus it is seen that the total concentration of the acid determines the intensity of the reaction. In other words the intensity of the reaction varies directly as the hydrogen ion concentration. This view is supported by comparing the ratings of the carbon dioxide and acetic and hydrochloric acid experiments. It is seen by comparing the carbon dioxide and acetic and hydrochloric acid experiments (see Table III.) that the rating on an average of the hydrochloric acid experiments are highest, acetic acid next and carbon dioxide lowest. This is due not to the higher molecular concentration of the hydrochloric acid over that of the acetic acid, since the molecular concentrations of the acetic acid were higher than that of the hydrochloric acid (Tables I. and II.), but to the higher ionization of hydrochloric acid over that of acetic acid and acetic acid over that of carbon dioxide in solution, thus giving higher ion concentrations.[1] These same

[1] Stieglitz's table of the ionization constants of acids, 1911.

points are suggested by the comparison of the ratings of each of the other three species in the hydrochloric acid, acetic acid and carbon dioxide experiments. It is interesting to note that when turnings only are considered that in all four species in the carbon dioxide, acetic acid and hydrochloric acid experiments that there is an increase of per cent. of turnings from the high acid concentration end over the low concentration acid end in the order named. Thus the hydrochloric acid again has the greatest intensity in avoidance of the high acid concentration end. These points are shown by the experiments with *propinquus* although the acetic acid experiments were of longer duration and were performed at a lower temperature than the hydrochloric acid experiments. The conclusion that intensity of negative reaction is directly proportional to the concentration of H ions can only be suggested, as sufficient data to warrant a definite conclusion are wanting.

The above suggestion might receive objection on the ground that there may be specific differences in the effect of the three acids used. Such an objection is supported by the fact that *propinquus*, in the low temperature experiment, is intoxicated by carbon dioxide and not by acetic acid. This apparent difference may, however, be explained on the ground that the carbon dioxide, since it diffuses more rapidly than the acetic acid, really produces a higher hydrogen ion concentration in the blood of the crayfishes than does the acetic acid, in spite of the fact that the latter acid is more highly ionized. The carbon dioxide would also tend to increase the carbon dioxide in the animal's blood by preventing the escape of the supply of this gas that is constantly being given off by the tissues of the animal. The acetic acid and hydrochloric acids would not offer any such hindrance to the diffusion of the internal gas into the water, and would not, therefore, be as detrimental as the carbon dioxide.

The periodicity and final cessation of invasion of the high concentration end of the experimental tank is a modification of behavior that may be brought about by increase in sensitiveness on the part of the crayfishes, or by a more rapid reaction to the same sensation. In the one case the cause is physiological, in the other the explanation must be psychological. If the

modification is psychological then the animals must respond from associated memory. Shelford and Allee ('13, '14) have pointed out that it is hard to locate the things associated. Besides association formation is usually very slow for Yerkes ('08) states that 50 to 100 trials are necessary for the crayfish to form a perfect association in a simple labyrinth. The same slowness of modification would be expected of association due to a stimulus unless the sensitiveness of the animals was in some way progressively increased.

The modification is rapid, the number of invasions being sometimes but one before complete avoidance of the high end followed. This modification is probably due, as Shelford and Allee ('13) have pointed out, to increased sensitiveness on the part of the crayfishes and as they have further suggested, the greater sensitiveness may be the result of an increase in the hydrogen ion content of the blood of the animals.

In the cases where *propinquus* came to rest upon the screen in the low end and remained there for the rest of the experiment, the reaction may be considered the climax of the behavior modification, especially since the animals made this reaction more quickly in the presence of high total concentrations of acid than in the low. These points are shown by *propinquus*, Chart I., Expts. 5, 14. The explanation as to why the animals came to rest at all after being made more sensitive by the acid is not clear, but probably a combination of factors, one of which is thigmotaxis, were acting.

Shelford and Allee ('13) suggest carbon dioxide as a factor in determining the distribution of fishes and that the same may be true for crayfishes is suggested by the foregoing experiments. Crayfishes react to very weak concentrations of carbon dioxide and acetic acid and they were not overcome by the carbon dioxide except in concentrations higher than usually appear in natural waters. There seems also to be a correlation of the specific reactions of the two species with their respective habits. *Propinquus* is a pond form and its reactions were directive, while *virilis*, a rapid stream form, gave reactions which were much less directive. In natural waters carbon dioxide would be encountered in rather high concentrations by *propinquus* and that this species may react to these concentrations to its own advan-

tage is indicated by the experiments heretofore described. *Virilis*, however, in its stream habitat would seldom encounter carbon dioxide concentrations of anything more than a very low degree and thus we find that this species does not react definitely to the gas. *Virilis*, then probably, while sensitive to carbon dioxide, orients itself in its environment by reactions to some other factor or factors. *Immunis* and *diogenes* are pond forms and are less sensitive to the acids but both avoid high concentrations. *Diogenes* although shown to be more sensitive than *immunis* orients less definitely as is shown by the per cent. of turnings from the high and low concentration acid ends. The specificities are again in coördination with the habitats of the species, *immunis* being a pond mud loving form, remaining in burrows only at times while *diogenes* remains almost wholly in burrows.

Summary.

1. Crayfishes sense the increase in carbon dioxide and acetic and hydrochloric acids in a gradient.

2. Both *propinquus* and *virilis* are intoxicated by carbon dioxide; *virilis* is also intoxicated by acetic acid but to a lesser degree.

3. The four species are susceptible to high concentrations of carbon dioxide and when subjected to high concentrations die in the following order, *virilis*, *propinquus*, *diogenes* and *immunis*.

4. *Propinquus* reacts negatively to the higher concentrations of carbon dioxide in a gradient, but when the total amount of acid present is large, the negative reaction may be interfered with by the direct detrimental effect of the acid.

5. *Virilis* reacts less definitely to the higher concentrations of carbon dioxide in a gradient than does *propinquus*. This is true whether the total concentration of the acid is large or small.

6. Both *diogenes* and *immunis* react more or less irregular to carbon dioxide due possibly to the lesser sensitiveness of these two species to this acid.

7. Both *propinquus* and *virilis* react negatively to the higher concentrations of acetic acid in gradients of this acid; *propinquus* reacts definitely in the presence of both high and low total concentrations; *virilis* reacts definitely to low total concentrations,

but not so definitely to high total concentrations; *diogenes* reacts irregular and less intense than the first two species while *immunis* reacts more definitely but with low intensity.

8. All four species react more strongly to hydrochloric acid than acetic acid and more strongly to acetic acid than carbon dioxide.

9. The intensity of avoiding reactions of all species to all acids tested as is shown by turnings only are in the following order; *propinquus*, *virilis*, *immunis* and *diogenes*.

10. The intensity of avoiding reactions of all four species varies directly as the total concentrations of the acids, and probably directly as the hydrogen ion concentration.

11. Rapid modification of behavior is shown by all four species. This modification may be due to the increased sensitiveness on the part of the animals, the increased sensitiveness being the result of higher ion concentration in the animal's blood.

12. The specific reactions of the crayfishes in gradients of carbon dioxide may be correlated with their habitats.

Acknowledgments.

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BREEDING HABITS OF THE HETERONEREIS FORM OF PLATYNEREIS MEGALOPS AT WOODS HOLE, MASS.

E. E. JUST.

Verrill ('73) first described *Platynereis megalops* figuring in his "Report" a male of the heteronereid phase. Later ('79) he figured the nereis-form and the female of the heteronereis-form changing the name he first gave, *Nectonereis megalops*, to *Nereis megalops*. Andrews, who ('91) had discovered the egg *Nereis limbata*, in a paper on the eyes of annelids speaks of the worm as *Nereis alacris*. I am indebted to Dr. J. Percy Moore who identified the animal as *Platynereis megalops*, Verrill. The belief seems to prevail that in the study of the cell lineage of *Nereis* ('92) Wilson indiscriminately used the males and females of *Nereis* and *Platynereis*. But this belief is by no means founded on any statement in Wilson's paper. Bonnevie ('08) has perhaps strengthened popular misconception through her descriptions of the "two varieties" of *Nereis limbata* at Woods Hole.

I. Swarming Habits.

The swarming of *Platynereis* is closely similar to that of *Nereis* (cf. Lillie and Just). There seems to be some variations as noted below. The behavior shows as that of many other forms a definite lunar periodicity: the sea-urchins (Tennent), the Japanese palolo (Izuka), the Pacific palolo (Woodworth and others), the Atlantic palolo (Mayer), *Amphitrite* (Scott), *Nereis dumerilii* (Hempelmann), etc.

Observations were made during the seasons of 1911, 1912 and 1913 at the Marine Biological Laboratory, Woods Hole, Mass. The swarming habits of *Platynereis* have not been worked out as fully as have been those of *Nereis limbata* (Lillie and Just, '13). In the first place during 1911 and 1912 attention was focused mainly on the swarming habits of *Nereis;* moreover, at all times the primary object in the collecting of *Platynereis* was for

experimental study. Strict watch, however, was kept on these worms throughout the summers named. This is especially true for the summer of 1913; during June, July, and August I went out every night, giving attention wholly to *Platynereis* swarming.

The animals on swarming nights swim near the surface of the sea, the males invariably appearing first, the females later. The females rarely exceed fifteen, and indeed on some nights no females swim, while the number of males may be very large. Verrill ('73) says that the worms swim at noon. I have never noted this.[1] Hempelmann ('11) might lead one to think that *Nereis dumerilii* swarms early in the morning. I looked for this during August, 1913, but did not find *Platynereis* swarming before or at sunrise. The evening swarm may last two hours.

The small reddish males swim with great rapidity in an ever more narrowing circle within the patch of light thrown by the observer's lantern until the swarm is at its height. Here and there often at a greater depth than the males swims with slow and even laborious movements, the larger female, pale yellow in color with a thin dorsal line of green—the remnant of the empty gut. One cannot but suspect that the sex ratio in some way depends on the rate of movement: the females are easy prey for fish, the males must easily escape their enemies. The sex ratio of the captured animals must be also influenced by the fact that the females tend to keep further below the surface than the males. This is true of *Autolytus* to a marked degree as I have repeatedly observed. (So too, Andrews, '92, and Mensch.) Verrill, however, says of *Nereis limbata* that in their burrows "there are few males in proportion to the females"—as in the case of *Platynereis*, the reverse is true of these worms during swarming.

As the male comes in the vicinity of female he swims very rapidly in spirals tangential to the surface. They swim together and after copulation and egg-laying, the female slowly sinks from view.

The swarming occurs nightly throughout the months of July and August during the dark of the moon. From new moon to full moon, whether there be moonlight or not the animals do not swarm. Only mature animals swarm.

[1] In July, 1914, I found spent males swimming during the day.

I have never taken this Heteronereid at Woods Hole earlier than June 29. In 1911 I remained at Woods Hole until September 18; I took no worms after August 24. For 1913, August 19 is the date of last capture.

The following tables selected from data of 1911, 1912 and 1913 give some idea of the lunar periodicity of the swarming:

TABLE I. 1911.

(Date of first capture, July 20.)

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	August 8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
	16	1	6
Third Quarter	17	0	0
	18	0	0
	19	0	0
	20	0	0
	21	4	5
	22	6	6
New Moon	23	8	8
	24	10	10
	25	0	0
	26	0	0
	27	0	0
	28	0	0

Comparison with *Nereis* shows in the first place that the number of worms swarming is not so great. It was found, for instance, in collecting *Nereis* to be practically impossible to make an accurate estimate of the number of males; for that reason a record was kept of the females only. On two or three nights only did I find it impossible to estimate the number of *Platynereis* males swarming; on other evenings it was easily possible to count them. The swarm of males on the evening of August 11, 1912, was wonderful. For a few minutes the sea was alive with thousands of the rapidly swimming Heteronereids. In 1913 there was a similar swarm of females, but in no such numbers. As in the case of *Nereis* the collections were made in one place during the three years.

The season, moreover, appears to be shorter than that of

TABLE II. 1912.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 4 to July 2	None	None
	July 3	0	1
	4	0	0
	5	0	0
	6	0	0
Third Quarter	July 7	0	0
	8	0	0
	9	0	0
	10	1	3
	11	3	3
	12	3	3
	13	0	2
New Moon	14	2	2
	15	0	2
	16	0	1
	17	0	0
	18	0	0
	19	0	0
	20	0	0
First Quarter	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	2	1
	26	1	0
	27	0	1
Full moon	28	0	0
	29	0	0
	30	15	30
	31	2	30
	August 1	4	30
	2	3	30
	3	0	20
	4	0	0
Third Quarter	5	8	20
	6	14	20
	7	0	0
	8	12	10
	9	10	50
	10	12	100's
	11	15	1000's
New Moon	12	18	30
	13	0	0
	14	0	0
	15	3	6
	16	0	0
	17	0	0
	18	0	0
First Quarter	19	0	0
	20	0	0
	21	0	0
	22	0	0
	23	0	0
	24	0	0
	25	0	0
	26	0	0

TABLE II. 1912.—*Continued.*

Moon Phase.	Date.	Number of Females.	Number of Males.
Full moon	27	0	1
	28	0	1
	29	0	0
	30	0	0
	31	0	0

TABLE III. 1913.

Moon Phase.	Date.	Number of Females.	Number of Males.
	June 13–28	None	None
	29	0	1
	30	0	0
	July 2	0	1
		None	None
	17	0	1
Full moon	18	0	10
	19	0	30
	20	0	20
	21	1	25
	22	0	10
	23	0	8
	24	2	4
	25	50	30
Third Quarter	26	2	4
	27	5	3
	28	16	20
	29	5	8
	30	20	16
	31	21	8
	August 1	6	6
New Moon	2	1	20
	3	4	25
	4	0	16
	5	0	0
	6	0	1
	7	0	0
First Quarter	8	0	0
	9	0	0
	10	0	0
	11	0	0
	12	0	0
	13	0	0
	14	0	0
	15	0	0
Full Moon	16	0	2
	17	1	0
	18	0	1
	19	0	2
	20	0	0
	21	0	0

Nereis. In this my observations approximate those of Verrill. Also, the yearly swarming shows more variations than that of

Nereis. This is strikingly brought out by a study of the tables—especially when one recalls that I gave attention wholly to *Platynereis* for the year 1913. Curves of the runs of *Platynereis* would show that the heights tend to fall in with those of *Nereis*. The lunar periodicity is therefore more like that of *Nereis limbata* than that of *N. dumerilii* which in some respects *Platynereis* resembles.

II. Egg-Laying.

Males and females caught with a hand-net in the evening at the surface of the water and kept in separate dishes may be studied in the laboratory. If a male be transferred with a female to a dish of clean sea-water, the phenomena observed in the sea may be readily followed. The female packed eggs discernible through her pale thin body wall swims slowly in a straight line; or, with head bent at right angles to the body describes a circle of which the head is the center. The male swims in spirals tangential to the surface of the water. Soon his spirals are along the course of the female, her body finally becoming the long axis of his helical body. He entwines the female through this performance and straightens out, thus clutching her in the twist of his body. If this embrace be in the posterior region of the female's body, the male loosens slightly and pulls himself along the female's body. The task appears to be exacting. Often I have observed a rather small male that had worked himself forward after having grasped an unusually large female near the anal segment fall apparently too exhausted to complete the courtship. As the male slips along forward over the female, he lashes his tail back and forth. The female bends her head as if seeking the tail. If the female keep her body in a straight line, the male must move anteriorly until he entwines her body in the pharyngeal region. He now forms a coil around her head of which his tail is the apex. He thrusts his tail down into the coil of his own body and so into the waiting jaws of the female. The female is quiescent throughout. About six seconds after the female has received the anal segment of the male, the animals separate and eggs stream from the posterior segments of the female. The male may be held for a time by the female; if so he swims around, dragging her. I believe that the eggs

escape, not through gonopores or the like, but through lesions of the body wall (cf. Scott.) Eggs escape from three or more posterior segments, occasionally from anterior segments. If escape by way of the posterior segments be experimentally inhibited, or if the female be slightly disturbed, the eggs seem to burst through the body wall at segments more anterior than otherwise. Females killed at the moment of oviposition show tears in the body wall.

After oviposition—and the whole process just described is in general the event of ten seconds—the female sinks to the bottom of the dish, a mere shred. In the laboratory placed in a little water it remains an irritable sticky mass for a time—incapable of exciting fresh males and finally dies, greatly shrivelled and blackened. Often, however, if flooded with fresh sea water it revives, expands to previous size, and swims around actively, almost perfectly transparent. I have kept these spent females alive for several hours. Since there are no sexual segments as in some annelids, but the whole body is little more than a locomotor ovary, it seems safe to assume that this egg-laying marks the end of the worm's existence.

Both animals must be in healthy condition for this behavior. Active males sometimes grasp females which because of rough handling in capturing are doubtless weak and fail to respond. The active males on the other hand are not very hardy: in the laboratory they rarely live twenty-four hours; one experiment made in 1913, failed to show any difference in the vitality of spent and unspent males. Normal females when placed in dishes with males fail to complete the courtship if the vitality of the male as by rough handling be impaired. Males and females may be kept in the same dish until death; if there be no courtship, there is no oviposition. Female *Platyneris* and male *Nereis* show no excitement when in the same dish, so male *Platynereis* and female *Nereis*. The male *Platynereis* ordinarily will embrace only an unspent female *Platynereis*. But on one occasion (July 23, 1913) all (8) males captured in turn and repeatedly embraced a *Nereis virens* eight inches long whose posterior segments had been lost. Once only I saw a male clutch a female which had extruded part of her eggs after a previous courtship.

The animals will go through this courtship when placed in a

very dense suspension of India ink in sea-water; or total darkness. The reaction, therefore, cannot be due to sight. It is more likely due to some chemical emanation from the gravid female only since the spent female is not attractive to the male (Cf. F. R. Lillie on *Nereis*, '12, '13.)

A male *Platynereis* will embrace at least four females. On the evening of August 24, 1911, for instance, I put a male and a female in a dish. They swam around for a time, then the male wrapped himself about the female just back of the head, he let go, uncoiled himself, his tail remaining in the female's mouth. Immediately after release, he was placed with a second female; a minute later he induced oviposition. After intervals of five minutes he embraced a third and fourth female. In all cases the worms shed eggs. The male placed in fresh sea-water with an active female after an hour (11 P.M., about two hours in the laboratory after capture) failed to make a fifth clutch. Other males embraced two females. During 1912 and 1913 these observations were verified.

If after this egg laying behavior, both animals be removed from the dish or if the eggs be pipetted off as laid the eggs develop and normal swimming larvæ much like those of *Nereis limbata* result. If at the moment of her release by the male the female be put in a dish of clean fresh sea-water, eggs will stream out and subsequently develop.

In all these cases sperm are attached to the vitelline membrane within a hull of jelly which has been secreted through the breakdown of the cortical protoplasm of the egg. As in *Nereis* this jelly formation begins at the moment that the sperm touches the membrane. In *Platynereis* it is easily demonstrated that the inseminated eggs have this jelly when laid. Mechanical pressure either by the male, experimentally, or otherwise, as has been repeatedly demonstrated, will not induce oviposition. Mere clutching however recurrent—even by more than one male is not sufficient stimulus for oviposition. The head of the worm may be crushed—eggs will not escape; if she be cut in two, a few eggs escape. Only after thorough drying on filter paper or on sheer dry linen will the eggs burst through the body wall. If the female be finely minced in sea-water practically all the eggs may

be procured. But eggs got in this way do not develop after insemination; they will not fertilize in sea-water. I have sections of uninseminated eggs killed after having remained upwards of two hours in sea-water; the cortical layer and the germinal vesicle are intact. (So *Nereis*.)

Eggs removed from worms after clutching only have the appearance of eggs from unembraced females—no sperm attached, subsequently no trace of development. Sperm are not found on the female's body (*e. g.*, hypodermic impregnation: cf. Whitman, Gardiner, etc.) or near the anus at the time of egg extrusion.

It appears, therefore, that mechanical stimulus is not sufficient to excite oviposition or sperm shedding. The eggs are not laid during or after the embrace nor are sperm shed unless the male's tail has been in the female's jaws. This, then, is a case of copulation followed by internal insemination. And indeed, the very elaborate and precise behavior indicates this. The sperm swallowed by the female inseminate the eggs in the body cavity, oviposition following immediately.

In 1911 gravid females before and after copulation were killed in Meves fluid but proved too refractory for cutting; in 1912, special precautions were taken. The following fixatives were used: Bouin, Gilson, 10 per cent. formalin, and Hennings mixture. With these mixtures the yolk and oil of the eggs are dissolved out, but the chitin of the jaws still makes the procuring of good sections difficult. In 1912 I thought that I had solved the difficulty when after experiments with various agents I procured with KCl, and KCN in sea-water eversion of the pharynx. But in 1913, these methods gave very indifferent results. Dissection of the jaws gave almost negative results. My best sections are those of July, 1912, killed in Gilson, Series A; those of August, 1912, killed in formalin, Series B, and those of 1913 kept in formalin for five months.

Sections of gravid females killed before courtship show no sperm in the body cavity. Sections of gravid females just after copulation show sperm among the antennæ, in the mouth, in the pharynx, and in the body cavity. The sperm may be traced, therefore, entering the mouth, passing down the pharynx whence they escape through lesions in the pharyngeal wall to the cœlom.

They may be found also attached to the vitelline membrane of the eggs. If one minces a male, one procures not only sperm but large numbers of corpuscles. Apparently, these are not injected into the female's body (cf. Scott on *Amphitrite*).

Since the mechanical pressure of the male, though often repeated, is not sufficient stimulus for egg-laying, it may be assumed that either the sperm or some secretion with, or of them stimulates in the female movements which bring about oviposition. In some cases males after having induced oviposition in two or three females cause egg-laying in a third or fourth as noted above. A slight amount of this substance, therefore if such there be in addition to the sperm themselves, is sufficient to initiate egg-laying. The injected substance, on the same ground could scarcely exert sufficient pressure to stimulate oviposition.

I had projected for 1913 various experiments to determine this point. The first experiment on the list, however, was clear enough to warrant abandoning the others. I put a female in a dish with no water. If a drop of sea-water be put on her head there is no response. Only complete drying causes breakdown of body wall. If instead of pure sea-water the minced female be added there is no response. But if a drop of minced male be added oviposition follows. This observation was made several times.

The following protocol from notes of the night of July 25, 1913, is typical:

Experiment.—Six males cut up in water adherent to their bodies (*i. e.*, not dried). Dried female put in this sperm suspension. No oviposition. A second female placed in the sperm suspension; and a third. No oviposition.

2. Three males cut up in three drops of sea-water. Two successive females used. No oviposition.

3. Six dried males cut up. Two dried females placed with heads in the sperm suspension. Eggs laid. Next day: trochophores.

4. Three males cut up in two drops of sea-water. Two dried females placed with heads in the sperm suspension. (Both females later copulated with males and laid eggs.) No eggs laid.

Oviposition, then, is clearly brought on through the ingesting of sperm with very little sea-water.

Nereis diversicolor O. F. Muller gives birth to living young. *Autolytus* (Agassiz) carries its larvæ in a brood pouch. In both of these forms there is probably internal insemination. Eisig

has described copulation in an annelid, *Capitella*. *Platynereis* is of interest in that oviposition so quickly follows copulation.

III. The Nereis Form of Platynereis Megalops.

As in the case of *Nereis* an attempt has been made to rear the larvæ of *Platynereis*. Best results were obtained during 1913. A table was kept of the development of the young worms and their characteristics noted. They closely resemble the larvæ of *Nereis dumerilii* described by Hempelmann which he obtained from his cultures. Some of my worms aged six months measured four centimeters. It is hoped that a study of these forms will give a clue to the swarming habit.

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ON THE STRUCTURE OF THE INNER EAR IN TWO PRIMITIVE REPTILES.

E. C. CASE.

In 1885 Cope[1] described the structure of the brain and the inner ear of one of the cotylosaurian reptiles, *Diadectes sp.* In this paper he figured the structure of the canals and concluded as follows: "The result of this examination into the structure of the auditory organs in the Diadectidæ may be stated as follows: The semicircular canals have the structure common to all the Gnathostomatous Chordata. The internal wall of the vestibule remains unossified as in many of the fishes and a few batrachians. There is no rudiment of the cochlea, but the vestibule is produced outward and upward to the fenestra ovalis in a way unknown in any other family of the vertebrates."

Fig. 1 shows the arrangement of the semicircular canals as given by Cope.

Among the many specimens collected from the Brier Creek Bone-bed by the University of Michigan expedition to Archer County, Texas, in the summer of 1913 there are several complete and nearly complete basi-cranial regions of the Permian or Permo-Carboniferous reptiles, *Dimetrodon* and *Edaphosaurus*. The structure of the basi-cranial region in these forms has already been described by the author,[2] but the recovery of this new material makes it possible to describe the condition of the ear cavity.

The specimen of *Edaphosaurus*, No. 3446 University of Michigan, probably belongs to the species *cruciger* of Cope. The bones of the ear region are undistorted and the cavity shares in the perfection of preservation. This is shown by the complete correspondence of the two sides and by the similarity of the cavity to that in less perfectly preserved specimens. If the co-

[1] Cope, *Proc. Am. Phil. Soc.*, p. 234, 1885.

[2] Case, Publication 55, Carnegie Institute of Washington, p. 98, 1907. Williston and Case, Publication 181, Carnegie Institution of Washington, p. 81, 1912.

ossified elements which shelter the inner ear be held in the normal position, as shown in Fig. 2, it will be seen that there is the trace of a groove running apparently horizontally from without inward; at the inner upper corner of the cavity it joins the trace of a second groove running nearly vertically. These are the marks of the anterior and posterior semicircular canals and correspond to the horizontal antero-posterior and the vertical antero-

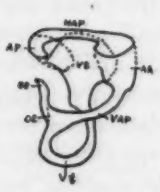

FIG. 1.

FIG. 2.

FIG. 1. Outline of the canals of the inner ear of *Diadectes* sp. From Cope. *hap*, horizontal antero-posterior canal; *aa*, anterior ampulla; *vap*, vertical antero-posterior canal; *vt*, vertical transverse canal; *ap*, posterior ampulla; *cc*, canalis communis of the vertical antero-posterior and the vertical transverse canals; *oc.os*, commune of the same.

FIG. 2. Inner view of the right otic region of *Edaphosaurus*, showing the cavity of the ear. *So*, supraoccipital; *op*, opisthotic; *fm*, foramen magnum. × 1.

posterior canals of Cope's figure. Where the two canals meet there is a projection upward and forward of the cavity which probably lodged a rudiment of the apex of the sinus utriculus superior (terminology of Retzius). At the outer ends of the canal there is evidence of slight enlargements which lodged the anterior and posterior ampullæ. Just below the point of union of the two canals there is an elevation of the cavity indicating that the sinus utriculus posterior was inclined inward and forward. The cavity for the utriculus is relatively large and higher than wide. On the outer side of the broken surface of the cavity, nearly opposite the middle point there is a slight excavation indicating the position of the external canal. At the lower end of the cavity there are two extensions, one running into the paroccipital (opisthotic) bone and parallel to its axis, this lodged the

lagena and shows no evidence of any curvature; the second extension is smaller and connected with the lagena cavity, it can only be for a considerable remnant of the sacculus.

The inner wall of the otic cavity was incomplete and the fenestra ovalis opened about opposite the upper end of the lagena.

The second specimen, No. 3447 University of Michigan, is that of one of the smaller but undeterminable species of *Dimetrodon*. It is as well preserved as that of the *Edaphosaurus*, both sides being present and identical in appearance and the details of the structure further verified by other specimens. The general arrangement of the canals and cavities is the same as in *Edaphosaurus* but it is smaller. The evidence for the presence of a

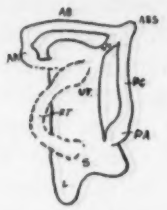

FIG. 3.

FIG. 4.

FIG. 3. Outline of the canals of the right side in *Edaphosaurus*. *ac*, anterior canal; *ass*, apex of the sinus urticule posterior; *pc*, posterior canal; *pa*, posterior ampulla; *s*, sacculus; *l*, lagena; *et*, external canal; *ut*, utriculus; *aa*, anterior ampulla. × 2.

FIG. 4. Outline of the canals of the right side in *Dimetrodon* sp. Lettering the same as in Fig. 3. × 2.

apex of the sinus utriculus superior is less pronounced than in the first specimen but there can be no doubt of its presence. The two lower cavities, for the lagena and the sacculus, are narrower and in less open connection. The arrangement of the canals is shown in Fig. 4.

The author is unable to bring the structure as here made out into adjustment with the figures given by Cope for *Diadectes*. In comparing the structure of the inner ear of these primitive forms with that of modern reptiles certain archaic characters are recognized. The presence of an apex to the sinus utriculus superior is notably primitive, Retzius shows it as present in certain fishes but it is not noted in the amphibians and reptiles.

The straight lagena and the relatively large remnant of the sacculus is also primitive; a large sacculus occurs in the Amphibia Ecaudata and in some lizards, as *Lacerta viridis*. The canals are entirely within the otic cavity and do not penetrate any of the adjacent bones. The lagena of the crocodile is quite elongate and the soft structures are said to show something of a twisting, the lagena of these forms is relatively shorter and broader than that of the crocodile but, of course, nothing can be said of the soft parts. The cavity of the inner ear does not exactly reproduce the membranous ear as the soft parts are separated from the wall by the perilymph but the author believes that the figures given represent a very fair approximation to the true form and proportions of the membranous ear.

PRELIMINARY REPORT OF CROSSING TWO HEMIPTEROUS SPECIES, WITH REFERENCE TO THE INHERITANCE OF A SECOND EXCLUSIVELY MALE CHARACTER.

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Attention of cytologists has been centered during the past few years on the chromosome theory that claims to offer an explanation of sex-determination—a theory due to the discovery of certain morphological differences in the chromosomes of the males and females of many species. This discovery is responsible for the chromosome hypothesis of sex-determination—an hypothesis that awaits the test of experiment.

Recently (Foot and Strobell, '13) we published the results of some cross-breeding experiments undertaken with the aim of testing the above mentioned hypothesis that the factors determining sex are carried and distributed by definite chromosomes. The character selected to test this hypothesis was an exclusively male character in *Euschistus variolarius*—a distinct dark spot which is present on the genital segment of the male only, and we claimed that the method of the transmission of this spot should be an index of the method of transmission of the entire male genital segment. This exclusively male character—the genital spot—is a distinguishing feature of *Euschistus variolarius*, but is absent in *E. servus*. We therefore selected these two species as well adapted to test the function of the so-called sex-chromosomes in the transmission of this exclusively male character. Our claim that the method of transmission of the genital spot may be interpreted as an index of the transmission of the male reproductive organs themselves has not been accepted by two critics (Morgan and Doncaster) who have recently referred to our results. In reply to the criticism of these two advocates of the chromosome theory of sex-determination, we quote the following from a paper now in press (Foot and Strobell, '14*b*).

"After this paper was sent to press a notice of our results appeared in the following publications: 'Heredity and Sex,' Morgan ('13), and 'Chromosomes, Heredity and Sex,' Doncaster, *Q. J. M. Sci.*, Vol. LIX. ('14).

The latter disposes of our results in a footnote, as data irrelevant to a paper entitled "Chromosomes, Heredity and Sex—A Review of the Present State of the Evidence with Regard to the Material Basis of Heredity, Transmission and Sex-Determination."

From his report of the evidence he draws the following conclusion:

"The facts of sex-limited[1] transmission thus support the hypothesis that both ordinary Mendelian factors and the sex-determining factor or factors are borne by chromosomes," p. 511, and in the above-mentioned footnote he adds: "The recently published work of Foot and Strobell cannot be used as an argument against this proposition. They have shown (as was previously known in birds and moths) that a secondary sexual character in Hemiptera can be transmitted through the sex that does not show it; but the character was not sex-limited[1] in transmission; their results, therefore, have no bearing in the present discussion." As opposed to this decision we claim that the very fact that the genital spot is not linked with one of the so-called sex chromosomes is a point that calls for a satisfactory explanation by those who believe in sex-determining chromosomes, and our results cannot be cancelled by a dogmatic assertion that they have no bearing on the subject.

Morgan treats the facts with more consideration and attempts to give an explanation of them, though his explanation appears to us more as an attempt to excuse the facts than to explain them. Part of his explanation is merely a restatement of our conclusions, and the remainder is not in harmony with the facts.

We concluded that our results demonstrate that the spot can be transmitted without the X- or the Y-chromosome and Morgan accepts this as follows, "these results may be explained on the assumption *that the factors lie in other chromosomes than the sex-chromosomes.*"[2]

[1] Sex-limited is used by Doncaster in the sense that sex-linked is used by Morgan.

[2] The italics are ours.

We concluded that if one assumes (for the sake of the argument) that the spot factors are in a diploid pair of chromosomes, it becomes necessary to assume other factors outside the chromosomes. We called such hypothetical factors "inhibiting factors" and we said of them: "We are forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes, in the region of pure hypothesis."

Morgan appears to accept this, calling such hypothetical factors "*things in the cell*," and symbolizing them as A. B. C. He says "the result (or character) that a factor produces depends on its relation to *other things in the cell* (here A. B. C.)," and he adds, "We are dealing, then, not with the relation of X to S *alone*, but this relation in turn depends on the proportion of both X and S to A. B. C."

In the above-quoted paragraph he includes in his explanation the assumption of a relation between the spot factors and the X-chromosome, and this we believe is a part of his explanation which is not sustained by the facts. The spot can be transmitted directly from the male to his male offspring—and therefore this must be by the male-producing spermatozoön—(if there is such a thing) and the so-called male-producing spermatozoön has no X-chromosome. It is impossible to believe that in such cases the inheritance of the spot is dependent upon the relation of the spot factors of the sperm to the X-chromosome in the egg, especially if the cross is made with a pure *servus* egg. Morgan evidently thinks this is possible, however, for in his explanatory diagram he illustrates a cross between *E. servus* ♀ × *E. variolarius* ♂, a cross which we explained we were unable to attempt on account of scarcity of material.

His diagram, if assumed to be an explanation based on the facts of our experiments, is further in error in its illustration of the F_1 hybrids. In his simple Mendelian scheme all the F_1 hybrids are illustrated as typical heterozygotes and the fact is ignored that two out of the eleven of our F_1 hybrids are like *servus* in having no spot. If such a modification of the spot can be caused by "*other things in the cell*," it would seem that merely calling these "A. B. C." is no explanation of the results.

Morgan excuses his attempted explanation on the ground

that we have failed to explain our results. We make no apology for this. We believe the duty of the scientist is to curb the natural temptation to force an explanation of individual results, for science to-day is overburdened by premature and undigested generalizations. We would aim rather to follow the example of those scientists who are willing patiently and conscientiously to collect data sustained by the hope that some day the facts may be of value.

Both Morgan and Doncaster class the genital spot of *variolarius* with the secondary sexual characters of authors and they therefore interpret our results as not having the bearing on the theories of sex-determination which we claim for them. Now our claim has been that the genital spot of *variolarius* is an integral part of the male genital segment—the structure of the female genital segment being such that the spot could not be present in this segment without changing the form of the segment itself—and we have claimed that therefore a study of the transmission of the genital spot should give a trustworthy indication of the method of transmission of the entire genital segment.

This claim that the method of transmission of the genital spot should be an index of the method of transmission of the genital organs of the male, has been completely justified by further work on these hybrids.

In the present paper we shall report the results from the study of the transmission of a second exclusively male character, and it seems to us that these results cannot be set aside as having no bearing on "Sex and Heredity," for this second exclusively male character is the male genital organ itself—the intromittent organ. The genetic results from our study of the genital spot of *variolarius* may be open to the criticism that as the spot is "not directly connected with the act of reproduction" it should be classed with the secondary sexual characters; but the intromittent organ is certainly free from such criticism and can be justly classed as a primary sexual character. In view of the fact that our results from the study of the transmission of the *variolarius* spot have been set aside on the ground that the spot is a secondary sexual character and therefore has no bearing on the problem of the determination of sex, it is necessary first

to establish the claim that the intromittent organ can be classed with the primary and not the secondary sexual characters. This apparently ought not to be difficult, but a difficulty does arise owing to the fact that recent authors who have discussed secondary sexual characters have avoided defining them and have neglected to state wherein they are to be distinguished from the primary sexual characters.

According to Darwin '59 Hunter defines secondary sexual characters as follows:

"The term, secondary sexual characters, used by Hunter, applies to characters which are attached to one sex; but are not directly connected with the act of reproduction."

Darwin '86 adopts Hunter's classification of primary and secondary sexual characters; but shows that even such an apparently clear cut definition encounters difficulties. He says:[1]

"With animals which have their sexes separated, the males necessarily differ from the females in their organs of reproduction; *and these afford the primary sexual characters.* But the sexes often differ in what Hunter has called secondary sexual characters, which are *not directly connected with the act of reproduction;* for instance, in the male possessing certain organs of sense or locomotion, of which the female is quite destitute, or in having them more highly-developed, in order that he may readily find or reach her; or again, in the male having special organs of prehension so as to hold her securely. These latter organs of infinitely diversified kinds graduate into, and in some cases can hardly be distinguished from, those which are commonly ranked as primary, such as the complex appendages at the apex of the abdomen in male insects. Unless indeed we confine the term 'primary' to the reproductive glands, it is scarcely possible to decide, as far as the organs of prehension are concerned, which ought to be called primary and which secondary" (p. 253).

Morgan '13 also appears to accept Hunter's classification, for in his rather full list of secondary sexual characters he includes none that are "directly connected with the act of reproduction." He opens his discussion of secondary sexual characters as follows:

[1] The italics are ours.

"The Secondary Sexual Characters."

"In the most highly evolved stages in the evolution of sex a new kind of character makes its appearance. This is the *secondary sexual character*. In most cases such characters are more elaborate in the male, but occasionally in the female. They are the most astonishing thing that nature has done: brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends" (p. 26).

If we are to discard Hunter's classification, because it is found difficult to determine into which class some of the characters rightly belong, we should have to be dissatisfied with many classifications that are thoroughly well established.

If we limit the term "primary sexual characters" to the reproductive glands, it offers an escape from the difficulties in classifying the prehension organs, as Darwin has pointed out; but it would seem that greater difficulties are met by refusing to place the intromittent organ in the same group with the reproductive glands; and placing it in the group with characters so far removed from "direct connection with the act of reproduction" as, for example, Morgan's list of secondary sexual characters, "brilliant colors, plumes, combs, wattles, and spurs, scent glands (pleasant and unpleasant); red spots, yellow spots, green spots, topknots and tails, horns, lanterns for the dark, songs, howlings, dances and tourneys—a medley of odds and ends." The intromittent organ is not only "directly connected with the act of reproduction"; but it is as much a part of the sex of the individual as the reproductive glands themselves. Any one of the characters in Morgan's entire list of male secondary sexual characters could appear in the female without changing her sex; but the intromittent organ is as clearly indicative of the sex as are the reproductive glands themselves.

If a definite chromosome carries the factors for determining sex and it therefore carries the factors for the reproductive glands, it would seem logical to suppose that the chromosome carrying the factors necessary for the development of the male reproductive glands would also carry the factors necessary for the

development of the intromittent organ which, when present, is functionally a necessary adjunct of the glands, and as indicative of the sex as the reproductive glands themselves. If we cannot accept the mode of transmission of the intromittent organ as an index of the mode of transmission of the reproductive glands, it would seem necessary to discard all structural features or other characters, which are distinctive of the gonads of a given species, such as their distinctions in size, form, color, etc., and assume that these characters, associated with the gland, have a different mode of transmission from the gland itself.

This would prevent any experimental test being applied to the chromosome theories of sex-determination and leave free scope for the wildest cytological speculations. If we should place the intromittent organ in the group of secondary sexual characters, because it has certain features in common with these characters we ought logically to place the reproductive glands themselves in the same group. For example, both these organs, in common with most of the secondary sexual characters, can be transmitted to the opposite sex—hermaphrodites appearing in forms that are normally sexually distinct. A case in point is Goodrichs's '12 interesting and important discovery of a male amphioxus in which 49 of the gonads were testes containing ripe spermatozoa and one was an ovary containing ripe ova. It may be urged that the intromittent organ is a secondary sexual character on the evidence that in the development of the embryo it appears much later than do the gonads—this indicating that the gonads are more fundamental and stable morphological entities. But there are facts opposed to this interpretation—Smith '10 found that when the spider crab is infected by the parasite sacculina, the testes can become so greatly metamorphosed that some of the cells may develop into ova and *the same testis* contain *both* ripe ova and spermatozoa.

It would seem that the division between primary and secondary sexual characters in common with almost all attempts at classification, has the objection that the line of demarcation is not, at all points, perfectly clear; but we believe, in spite of this, that we are justified in classing the intromittent organ as a primary sexual character and that the results from the study of the trans-

mission of this organ may justly be claimed as an index of the method of transmission of the reproductive glands themselves.

Before giving these results we would express our great indebtedness to Professor Poulton and to Dr. Eltringham, of Oxford. We are indebted to Professor Poulton for his kind response to our wish to find an experienced entomologist in England who would be willing to study *E. variolarius* and *E. servus* with the aim of finding other characters than the genital spot that could be studied in the hybrids. He kindly suggested Dr. Eltringham, of Oxford, to whom we are indebted for the discovery that there is a marked specific difference in the intromittent organ of *E. variolarius* and *E. servus*. This discovery has made it possible for us to secure the results which are recorded in this paper.

Results and Discussion.

The intromittent organs of *E. variolarius* and *E. servus* differ markedly in their length. We have dissected these organs from the genital segment of many of the parent species, and from all the hybrids, both of the F_1 and F_2 generations. These have been mounted and photographed at a magnification of 20 diameters, and all have been carefully measured at this magnification. The intromittent organ of *E. variolarius* varies in length between 85·5 and 106 mm., while that of *E. servus* varies between 146 and 182 mm. These measurements were made from 62 pure *variolarius* specimens, and from 62 pure *servus* specimens, the mean length of the intromittent organ of *variolarius* being 96·5 mm., and of *servus* 166·41 mm.[1]

Photos 1 to 4 show four typical intromittent organs of *E. variolarius*, these four varying in length between 94 mm. and 98·5 mm. Photos 5 to 8 show four typical intromittent organs from *E. servus*, these four varying in length between 158 mm. and 182 mm. Photos 9 to 12 show four typical intromittent organs of the F_1 generation derived from *E. variolarius* ♀ × *E. servus* ♂. These four vary in length from 122 to 132 mm. We have ten intromittent organs of this F_1 generation, nine of these being variable intermediates, and one like pure *variolarius*.

[1] A discussion of the mean lengths of the hybrids and of the back-cross, and their bearing on the Mendelian type of inheritance, will be given in a later and more detailed report of these results.

Thus the type of intromittent organ characteristic of the species is transmitted through the female to her male offspring, and also directly by the male, and we may add that this is further proved by the back cross (F_1 ♀ × pure *variolarius* ♂). Thus the mode of transmission of this second exclusively male character is like that of the genital spot—*both* of these exclusively male characters being transmitted through the female as well as directly from the male—neither of these characters therefore being sex-linked.

Photos 13 to 20 show eight typical intromittent organs from the F_2 generation, these eight varying in length between 85·5 mm. and 140 mm. A few of these specimens (photos 13–15) show that the factors which determine the genital spot and those which determine the intromittent organ are not linked in inheritance (see below).

In our study of the transmission of the genital spot of *variolarius* (Foot and Strobell, '14*a*) we divided the hybrids into three groups—those having a genital spot like that of pure *variolarius*, those without a spot like *servus*, and those with a spot intermediate between these two extremes. In order to compare the results from the study of the two exclusively male characters—the genital spot and the intromittent organ—we have again grouped the hybrids into three classes, those having a length of intromittent organ like that of *variolarius*, those with a length of organ like that of *servus*, and those with a length intermediate between these two extremes.

By this grouping it is possible to compare the genital spot of each individual hybrid with the type of intromittent organ of the same hybrid, in order to determine whether these two exclusively male characters are linked in inheritance—to determine to what extent the two are associated in their transmission. Before discussing this point we shall summarize the points of agreement in the inheritance of the two characters.

The intromittent organ—like the genital spot—is not sex-linked, this being shown by the facts that it is transmitted through the female, and also directly from the male to his male offspring. The intromittent organ—like the genital spot—is transmitted without the aid of either of the so-called "sex-chromosomes." It is transmitted without the aid of the Y-

chromosome because it is inherited through the female, and it is transmitted without the aid of the X-chromosome because it is transmitted *directly* from the male to his male offspring. Like the genital spot, the intromittent organ fails to show dominance in the F_1 generation, and fails to show a simple Mendelian ratio in the F_2 generation; but the details demonstrating these facts must be reserved for our full report of this work, in which it will be possible to compare the inheritance of these two exclusively male characters in every individual of the F_1 and F_2 generations.

As in the case of the genital spot, we are forced to conclude that if the factors determining the inheritance of the intromittent organ are carried by definite chromosomes, they must be in *at least* a pair of diploid chromosomes, and as in the case of the genital spot, we are further forced to conclude that there are factors in the cell, outside the chromosomes, which determine just how many of the factors determining the character of intromittent organ shall find expression in the first and second generations of hybrids. The facts show, as in the case of the genital spot, that this cannot be accomplished by the assumed mechanism of division of the chromosomes, but is dependent upon hypothetical factors outside the chromosomes, and thus the distribution of unit factors through the mechanism of chromosome division seems to be an unnecessary assumption. If the factors essential to produce these two exclusively male characters are confined to one chromosome they can be in the Y-chromosome *alone*, for according to the mechanism of the two maturation divisions this is the *only* chromosome that can be in *all* the so-called male-producing spermatozoa. The facts, however, demonstrate that not only the genital spot, but the intromittent organ, can be transmitted without the aid of the Y-chromosome. If, as the facts demand, the factors for these two exclusively male characters cannot be in less than a pair of chromosomes, there seems no adequate reason for confining them to a single pair, or even to the chromosomes at all, for if factors outside the chromosomes and outside the mechanism of the division of the chromosomes, are responsible for the exact expression or total suppression of these characters, this deprives the chromosomes of a most important function which has been attributed to them, based on the mechanism of their division.

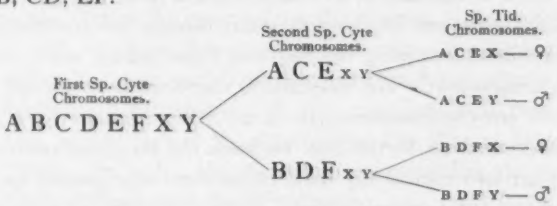
The results from the back cross demonstrate that the type of intromittent organ distinctive of the species can (like the presence or absence of the genital spot) be transmitted by *both* the so-called male-producing and female-producing spermatozoa. The back cross demonstrates not only that the male can directly transmit the intromittent organ distinctive of *variolarius;* but that the type of intromittent organ distinctive of *servus* is transmitted by the F_1 female, and therefore was transmitted by the female-producing spermatozoön of the first cross. If such a primary sexual character of the male can be carried by the female-producing spermatozoön, it is only logical to believe that female primary sexual characters also can be transmitted by both types of spermatozoa (male-producing as well as female-producing)—for it is difficult to believe that male and female primary sexual characters differ fundamentally in their method of transmission. The facts appear to deprive the male-producing spermatozoön of its distinctive function, and challenge the logic of endowing slight morphological differences in structures of the cell with causal attributes of fundamental importance.

Linkage in Inheritance.—If factors which stand for a given character are carried by a definite chromosome or pair of chromosomes, and the inheritance of the character is due to a special distribution of the factors at mitosis, it would seem logical to expect that the factors of two characters showing a very special mode of distribution (*i. e.*, exclusively male characters) would be contained in the same chromosome, and that this would be indicated by their being linked in the hybrids. We would expect the absence or presence of the genital spot, distinctive of one species, to be associated in inheritance with the type of intromittent organ characteristic of the same species. Even if the extent to which a character appears is dependent upon hypothetical factors outside the chromosomes, we would expect these hypothetical factors to act equally on two characters which are so closely associated as to be contained in the same chromosome. We should expect the two characters never to be so entirely dissociated that we find, in the same individual, the absence of spot characteristic of one species, associated with the type of intromittent organ distinctive of the other species. Instances of

such complete dissociation do however occur and are shown, for example, in photos 13 and 14, which have the length of intromittent organ characteristic of *E. variolarius* (85·5 mm. and 99 mm.) while the specimens from which these organs were dissected have the *E. servus* absence of spot. Photo 15 has a length of intromittent organ almost equal to *E. servus* (140 mm.), while the specimen from which this was dissected has the genital spot distinctive of *E. variolarius*. There are, however, instances of association in the inheritance of the two characters, the intromittent organ and genital spot typical of one of the species occurring in the same F_2 individual; but exact classification of the full results shows that the two characters are transmitted quite independently of each other. The intermediates, having a large range of variation, make it possible for many of them to appear to show the two characters in the association that would be in harmony with the chromosome hypothesis, but an exact comparison shows that two plus and two minus intermediates are quite as frequently associated as are a plus and a minus intermediate. If we find such independence in the transmission of the two characters, there seems no logical reason for assuming that their factors are carried by the same chromosome. If their frequent independence in transmission forces us to locate them in at least two of the seven chromosomes contributed by each parent, there seems no adequate reason for confining them to the chromosomes at all, especially as their final mode of expression is not dependent upon the distribution at mitosis of unit factors carried by the chromosomes, but upon hypothetical factors outside the chromosomes.

As the advocates of the sex-determination theory may dismiss these results—as in the case of the genital spot—on the ground that the intromittent organ is not sex-linked, and is merely a secondary sexual character, it may be profitable to attempt to follow theoretically the transmission of what must be admitted are *primary sexual* organs, (the ovaries and the testes), while assuming that they are "sex-linked." This has its difficulties in the case of the testes, for the factors determining the sex-linked characters of authors are assumed to be in the chromosome which is homozygous in one sex and heterozygous in the other

sex. This would place sex-linked factors for the testes in the X-chromosome of these insects; and locating these factors in this chromosome would involve their being in the female-producing spermatozoa only, and this type of spermatozoa therefore would carry the determining factors for *both* the ovaries and the testes, while the male-producing spermatozoa would carry neither the one nor the other. We shall try to show by an analysis of the chromosomes that it is as impossible to associate the testes with the sex-chromosomes as we have shown by experiment is true for the two other exclusively male characters—the genital spot and the intromittent organ. In order to simplify the discussion, we reproduce the following diagram used in an earlier paper to show the method of division of the 14 somatic chromosomes which are distinctive of both *E. variolarius* and *E. servus*. In the diagram we have used the method of designating univalents by the letters of the alphabet, bivalents being represented by AB, CD, EF.



Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY*-chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y*-chromosome.[1]

The above diagram demonstrates the two types of spermatozoa, those having the X-chromosome—so-called female-producing; and those having the Y-chromosome—so-called male-producing. If we accept Morgan's conclusion that factors determining all sex-linked characters are located in the X-chromosomes and we assume that the primary sexual characters are sex-linked, this involves placing the factors determining both the ovaries and the

[1] Only six of the twelve autosomes are designated.

testes in the X-chromosomes and, as stated above, this would effectually deprive the so-called male-producing spermatozoa of an essential male-producing function. This is so evidently out of harmony with the chromosome sex-determination theory, that it needs no further comment.

If we attempt to place the factors determining the testes in the other sex-chromosome (the Y-chromosome) we meet difficulties that are equally obvious, for there are many forms that have no Y-chromosome at all. If we could ignore this important fact, we would have, in these insects, quite a diagrammatic demonstration of the chromosome sex-determination theory, for the Y-chromosome is the *only* chromosome that is in *all* the male-producing spermatozoa, just as the X-chromosome is the *only* chromosome that is in *all* the female-producing spermatozoa. Each is the *only* chromosome which is distinctive of the type of spermatozoa which it identifies. But the fact cannot be ignored that the Y-chromosome, so conspicuous in these insects, is absent in most forms, and we must therefore dismiss the possibility that the factors determining the testes of these insects are carried by this chromosome. The association therefore between the testes and the sex-chromosomes can be no closer than we have shown by experiment to be the case between the sex chromosomes and the other two exclusively male characters—the genital spot and the intromittent organ.

Realizing that the Y-chromosome cannot logically function as the carrier of the factors determining a male, Morgan '11 suggested that "the factors for producing the male must be in some other chromosome" (than the Y- or the X-chromosomes). We would consider this suggestion in relation to the factors determining the testes of these insects, ignoring for the present the fact that in his diagram illustrating this suggestion, Morgan does not place these factors in *one* chromosome but in a pair of chromosomes. If we attempt to place the factors for the testes in one of the autosomes, we meet difficulties that are quite as obvious as the difficulties in attempting to place the factors in the sex-chromosomes.

The above diagram (text Fig. 1) shows that the spermatozoa can be classed not only into two types (the so-called male-produc-

ing and female-producing), but each of these groups can again be separated into two types, in relation to their autosome content —one type containing the autosomes A, C, E, and the other type the autosomes B, D, F. As we are discussing the transmission of the testes, the factors for which are presumably carried by the male-producing spermatozoa, we shall consider the two types of these spermatozoa only—those with A, C, E, Y, and those with B, D, F, Y.

Our problem, as stated above, is to determine whether it is possible to place the factors which determine the testes of these insects in one of the autosomes.

A glance at text Fig. 1 shows that each of the autosomes is in *only half* the male-producing spermatozoa, and is also in half the female-producing spermatozoa. If, for example, we assume that autosome A carries the factors for determining the testes, and the X-chromosome carries the factors for determining the ovaries, we shall have *all* the female-producing spermatozoa carrying the factors for determining the ovaries, and in addition to this, half of these spermatozoa will carry the factors for determining the testes, as half of them have the A autosome. The male-producing spermatozoa, on the contrary, will not only carry none of the factors for determining the ovaries, but only half of them can carry the factors for determining the testes, as only half of them have the A autosome.

These conclusions, forced by an analysis of the chromosomes, are by no means in harmony with the demands of the chromosome sex-determination theory—thus it is quite as impossible to confine the factors for the testes to a single autosome, as we have shown is the case with the other two exculsively male characters —the genital spot and the intromittent organ. We might avoid this difficulty by assuming that the maturation divisions of one pair of the autosomes is like that of the XY-chromosomes, and that the factors for the testes are carried by one member of this pair. This involves, however, the further assumption that this autosome must follow the lead of the Y-chromosome or it might arrive in the female-producing spermatozoön. Unless we are willing to make some such unwarranted assumptions, it does not seem possible to make the association between the testes and

the sex-chromosomes any closer than we have shown by experiment is the case between these chromosomes and the genital spot and intromittent organ. Those who would place these factors in the chromosomes must concede that they must be in *at least* a pair of autosomes; there seems indeed no reason for assuming a different mode of transmission for the testes than for the other exclusively male characters—the genital spot and the intromittent organ. We feel we are therefore justified in our claim that the mode of transmission of the genital spot and intromittent organ is an index of the mode of transmission of the reproductive glands themselves, and that our cross-breeding experiments offer direct evidence against the chromosome theory of sex-determination.

If we reconsider Morgan's suggestion that "the factors for producing a male must be located in some other chromosome" than the X-chromosome, and we interpret "some other chromosome" as a pair of autosomes (as Morgan does in his formula) this would locate the factors for the testes in this pair of autosomes and be quite in harmony with our conclusions, that factors for exclusively male organs, if carried by chromosomes, cannot be in less than a pair of chromosomes—and it supports our claim that the method of transmission of the genital spot and intromittent organ is an index of the method of transmission of the testes.

Morgan's formula

Gametes of female—X M—X M
Gametes of male—X M—M.

gives the female zygotes (X M + X M) just as many factors for "producing a male" (M M) as it gives for producing a female (X X) but he does not tell us what determines that the X gametes shall predominate.

According to Morgan's formula the term "female-producing spermatozoön" (X M) would appear to be a misnomer, for it carries "the factors for producing a male" as well as the factors for producing a female. The male-producing spermatozoön, on the contrary, carries the factors for producing a male only (M). The female-producing spermatozoön therefore can transmit exclusively male characters, as we have shown is the case, but

the male-producing spermatozoa cannot transmit exclusively female characters. That exclusively female characters and exclusively male characters should have such a different mode of transmission does not appear to us to be a logical conclusion, but it is a question that it is possible to put to the test of experiment.

The point of view of the investigator as to the chromosome theory of sex-determination seems to be entirely dependent upon the extent of his belief in the individuality of the chromosomes. In these insects, for example, the so-called male-producing spermatozoa have the Y-chromosome and not the X-chromosome, and those who believe in such a degree of individuality of the chromosomes as is demanded by the chromosome hypothesis of sex-determination, must hold that a so-called male-producing spermatozoön *must* develop into a pronucleus with a Y-chromosome and never an X-chromosome. They must hold that the Y- and X-chromosomes are as individual as the king and queen of chess for example.

On the other hand, the cytologist who believes that the chromosomes, like other organs in the cell, are the expression rather than the cause of cell activities, can also believe that there are forces outside the chromosomes that determine whether an egg shall develop into a male or female and can further believe that these forces, acting on the developing pronucleus can cause its chromatin content to develop into the chromosome configuration which is demanded by the sex. As it is impossible to follow the metamorphosis of a spermatozoön into a pronucleus, the cytological proof can probably never be achieved; but there is definite evidence that cells which normally produce certain organs, can be forced by experimental manipulation to produce other organs which have quite different functions, and such a change of function must create a corresponding change of structure, not only in the visible final result, but in the initial changes of the cell itself. Thus we believe that the structure of the cell, or any part of the cell is not the determining factor, but is merely an expression of other forces.

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Description of Plate I.

The length of the intromittent organs was measured by a small pair of architect's dividers fitted with No. 9 needle points, and the dividers were frequently tested by measuring a 100 mm. line. Each intromittent organ was photographed at a magnification of 20 diameters, and the measuring was simplified by numbering each division of 20 mm. by a pencil mark. The measurements were taken from the distal end of the intromittent organ at the point where the coil enters the gland, the coil being easily dissected off at this point (photos 3, 4, etc.).

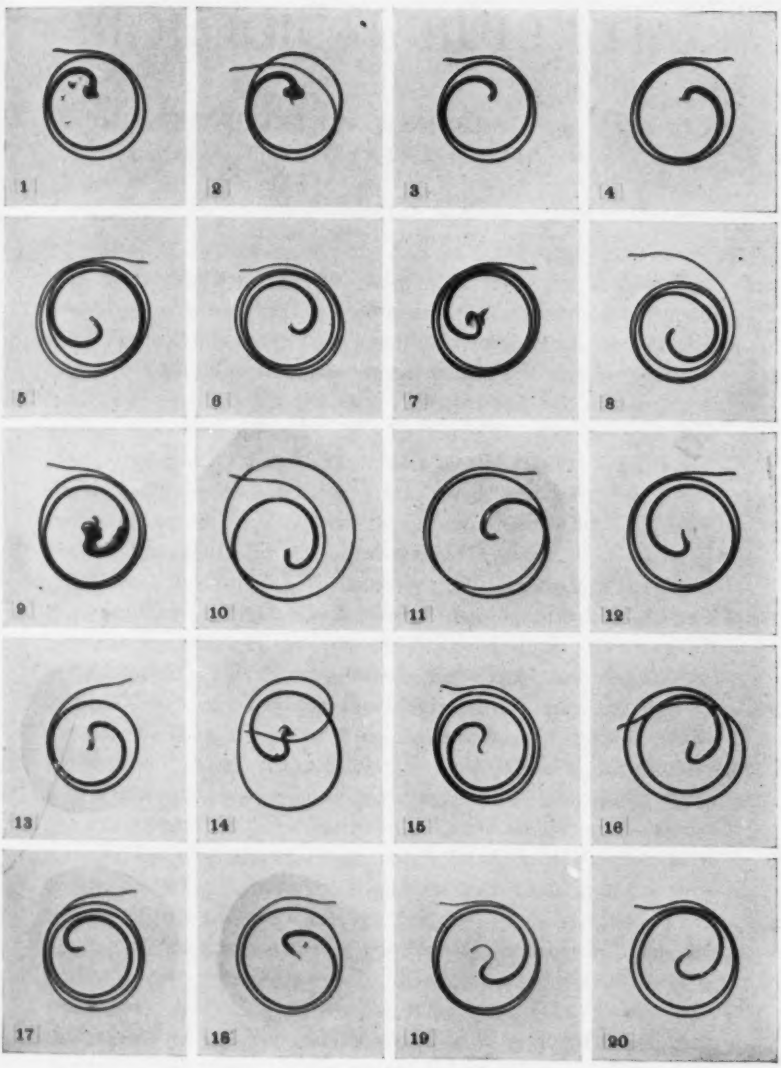
In photos 1, 2, 7, 9, a small part of the gland itself is retained. When part of the intromittent organ that is within the gland is preserved, the point from which the measurement was taken is easily determined, for the part within the gland is transparent and tapers at this point to a much smaller canal, *e. g.*, photos 15 and 19.

Photos 1 to 4. Typical specimens of the intromittent organ of *Euschistus variolarius*. Length of the organ of photo 1, 98·5 mm. Photo 2, length 97·5 mm. Photo 3, length 95 mm. Photo 4, length 94 mm.

Photos 5 to 8. Typical specimens of the intromittent organ of *E. servus*. Length of the organ of photo 5, 182 mm. Photo 6, length 158 mm. Photo 7, length 170 mm. Photo 8, length 162 mm.

Photos 9 to 12. The intromittent organs from four of the F_1 hybrids. Length of the organ of photo 9, 124 mm. Photo 10, length 132 mm. Photo 11, length 122 mm. Photo 12, length 126 mm.

Photos 13 to 20. The intromittent organs from eight of the F_2 generation. Length of the organ of photo 13, 85·5 mm. Photo 14, length 97 mm. Photo 15, length 140 mm. Photo 16, length 128 mm. Photo 17, length 147 mm. Photo 18, length 100 mm. Photo 19, length 124 mm. Photo 20, length 124 mm.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